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Biomonitoring for Control of Toxicity in Effluent Discharges to the Marine Environment

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Biomonitoring for Control of Toxicity in Effluent Discharges to the Marine Environment

September 1989

Center for Environmental Research Information
Office of Research and Development
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Environmental Research Laboratory
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U.S. Environmental Protection Agency
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Foreword

This document describes the use of biological monitoring as an effective water quality -based approach to controlling the toxicity in discharges to estuarine and marine waters. The development of these methods was conducted primarily at EPA's Environmental Research Laboratory in Narragansett, Rhode Island (ERL-N). This document is intended for Federal and State National Pollutant Discharge Elimination System (NPDES) permit writers, water quality specialists, managers of pollution control systems. regulatory decisionmakers, research scientists, and informed laypersons.

The document is organized into seven sections. Section 1, Introduction, is an overview of the regulations that mandate a change to water quality-based permit limits and the testing programs that support that change. Section 2, Marine/Estuarine Complex Effluent Toxicity Testing Program (CETTP), describes EPA's efforts to establish the validity of whole-effluent toxicity testing for marine/estuarine environments. The section describes the test methods developed to determine the effects of whole effluents on survival, growth, and reproduction of several test species. Section 3, In Situ Biomonitoring, presents a program to determine the biological responses of marine and estuarine test species to pollutants. Section 4, Integration of Effluent and In Situ Biomonitoring Programs, describes how the complex effluent and in situ biomonitoring programs may be used in tandem to control toxic discharges to marine waters. Section 5, Application of the Biomonitoring Strategy: New Bedford Harbor Pilot Dredging Program, describes how ERL-N employed the tandem approach described in the previous section to monitor cleanup options for one of the most polluted industrial harbors in the country. Section 6, Conclusions and Future Directions, discusses possible refinements to existing marine biomonitoring programs and their use in NPDES permitting. Appendix A summarizes the results of five case studies in which the CETTP and in situ biomonitoring programs were evaluated, and Appendix B is a glossary of terms useful in understanding the monitoring programs.

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1. Introduction

Regulatory Background

In 1972, the National Pollutant Discharge Elimination System (NPDES) was established under the Clean Water Act to control point source pollution to the Nation's surface waters. Point sources of pollution can be directly attributed to a specific source, such as a factory or publicly owned treatment works. Every point source must apply for and obtain an NPDES permit that limits the concentrations of pollutants that may be discharged in its effluent. EPA has delegated permit-setting activities to States whose permit programs meet applicable EPA requirements; these States operate their own NPDES programs with EPA overview.

In 1984, EPA issued a policy statement recommending *effluent biomonitoring* as one part of a "water quality-based approach to controlling toxic pollutants" (1). The policy directed States to use data from biological testing of complex effluents discharged from point sources for setting NPDES permit limits. EPA also prepared a technical support document (2) that described ways to implement the policy. This policy made biological testing an integral part of the NPDES program under the Clean Water Act, and effectively changed the permit-setting emphasis from a technology-based approach to a water quality-based approach. The 1987 amendments to the Clean Water Act (now called the Water Quality Act) reinforced this policy by stating that water quality standards should be chemical-specific numbers when such numbers have been established, however biological monitoring should be used in areas where no criteria for aquatic life exist and to address the effect that the interaction of chemicals has on toxicity (3). EPA made a more definite step toward water quality-based control in a proposed rule published in the *Federal Register* in January, 1989 (4), which states that "controls for whole effluent toxicity are an essential component of EPA's integrated approach to toxics control."

To control the discharge of toxics under the toxicity-based approach, EPA recommends that complex effluent limits be used in conjunction with limits on specific pollutants. In many cases aquatic toxicity data for specific chemical compounds are often not available for use in setting standards. Also, wastewaters usually contain mixtures of chemicals that are difficult for industries or municipalities to characterize. Compounding these problems is the fact that even if pollutants are discharged at toxic concentrations below analytical detection limits, the effluent may still be toxic. This toxicity may be due to a variety of factors: an excess of nonconventional pollutants (such as total suspended solids); conventional pollutants; unanticipated interactions between the chemical mixtures in the effluent; and/or unanticipated interactions between the effluent and the receiving water. Complex effluent toxicity testing takes these factors into account and complements the chemical-by-chemical approach.

Special Considerations for Marine and Estuarine Waters

Since the 1984 shift to a water quality-based approach, biomonitoring methods in freshwaters such as lakes and rivers have been well established. Translating these methods from freshwater to marine environments, however, is not a simple task. Differences between the two systems require alternative testing methods and test species. In addition, the physical differences between streams and estuaries, rivers and bays, as well as the chemical differences between saltwater and freshwater, require special consideration.

Physical Differences

Many physical conditions affect the mixing of effluents in marine/estuarine and freshwater systems. Marine and estuarine systems, however, are much more complex - water depth and current direction vary with the tides, and tidal volume varies on a monthly and seasonal basis. Models that predict effluent mixing must take these and other factors into account. For example, an outgoing tide may quickly carry the effluent plume into deeper waters, but if little vertical or lateral mixing occurs along the plume, then a concentrated effluent could be delivered to a critical resource area (oyster beds, spawning grounds, etc.). In addition, estuaries tend to act as chemical "sinks." Stratification can trap effluents below horizontal temperature gradients in the water, and the tide's oscillation can restrict the plume from significant horizontal movement (2). The contaminants sit and collect in what becomes a basin for pollution. Finally, effluent plumes may mix or disperse less easily in saltwater than in freshwater because of differences in salinity and specific gravity between the effluent and the surrounding waters.

Chemical Differences

The buffering capacity of saltwater is much greater than that of freshwater. Certain chemicals either break down more easily or remain in their original toxic form longer in saltwater than they would in freshwater. Also, the speciation or form of the individual chemical mixture components (e.g., solid, liquid, ion, or complexed) varies depending on whether the effluent is in saltwater or

freshwater. This is an important factor because some chemicals are accumulated or degraded by organisms in one form, but not in another.

Biological Differences

Marine and estuarine waters, in general, support a greater diversity of species than do freshwater rivers and lakes. In addition, unlike rivers and lakes, in which fish may spawn and live the rest of their lives in one area, estuaries and bays act as nursery grounds for many species that later move out into open waters. Exposure to toxic effluents is more likely to affect these immature, more sensitive life stages.

Resulting Differences in Approach

These physical, chemical, and biological differences between marine and freshwater environments require different effluent and organism sampling methods as well. Because of a marine or estuarine water body's depth and expanse, and the possibility of disturbances from violent storms, marine biomonitoring logistics are more difficult to plan and carry out. In freshwater biomonitoring, benthic surveys are frequently used. These surveys involve cataloging all of the species inhabiting the benthos, and characterizing the "health" of the water body on that basis. However, the benthic zone in marine environments often may not be directly affected by toxic discharges. The discharge, if released in a freshwater plume, flows up through the water column to the surface of the water and usually does not flow along the bottom. Suspended solids, however, may settle to the bottom. Furthermore, findings from biological surveys of benthic species may indicate residual effects from past pollution. Estuaries tend to serve as depositional areas, therefore many discharges, particularly municipal discharges, superficially enrich the benthic sediments with organic carbon. This enrichment by itself, in the absence of toxic chemical contamination, can substantially alter the biological community.

Populations of species within the marine community also change naturally as they move, die out, or populate the benthos in a patchy or irregular fashion, making the detection of adverse effects from pollutants more difficult. The ambient receiving water toxicity tests used in marine and estuarine environments measure the effects of the suspended plume on indigenous species. In situ biomonitoring methods provide another alternative for measuring the effects of effluents on marine species. This permits the effects of any toxicity present in the waters surrounding the discharge to be measured directly, without relying on laboratory exposure systems.

The great diversity in species present in the marine environment affects species selection for toxicity testing as well. Animal and plant kingdoms both must be represented in testing. All of these factors were considered when adapting freshwater methodologies to marine conditions and species.

Complex Effluent Toxicity Testing Program

The Complex Effluent Toxicity Testing Program (CETTP) was initiated as a means of testing whole effluents using aquatic organisms as indicators of effect. The overall goal of the CETTP was to enable regulators to use the parameter toxicity data to set NPDES permit limits based on water quality protection. Researchers first applied the technology to freshwater systems. From the program's outset in 1982, EPA has studied the toxic effects of effluents in several rivers in the United States, with five goals in mind:

- To verify that complex effluent toxicity tests predict and quantify adverse impacts to receiving waters.
- To identify complex effluent toxicity test procedures that support the NPDES regulatory process.
- To demonstrate, through case studies, the effectiveness of tests in a variety of discharge scenarios.
- To field test short-term chronic toxicity tests.
- To compare the receiving water toxicity with the effluent toxicity observed at known concentrations (5).

The freshwater CETTP resulted in several biological tests that established the validity of whole effluent toxicity testing.

EPA's marine CETTP set out to achieve the same five goals described above for the freshwater portion of the CETTP. To adequately address the differences between freshwater and marine/estuarine water systems, the number of test species was expanded, and ambient receiving water toxicity tests were used rather than biosurveys. The biomonitoring tests described here were designed for the measurement of toxicity to the environment where relatively small ratios of effluent volume to receiving water volume exist. Freshwater receiving streams may contain more than 60 percent effluent after discharge, but most estuaries and bays, by virtue of their potentially greater volume, generally provide higher effluent dilution.

In Situ Marine and Estuarine Biomonitoring Program

The in situ marine biomonitoring program focuses on determining not only contaminant concentrations, but also contaminant effects. This program extends the CETTP policy of emphasizing specific effects rather than specific chemicals. The five objectives of the program are:

- To identify general problem areas or conditions of the Nation's bays and estuaries and other near-coastal waters.
- To track dischargers' response to regulations by monitoring point source discharges and nonpoint sources of pollution.
- To identify links between chemical pollutants and biological responses.
- To identify links between impacts at different levels of biological organization.
- To predict population- and community-level effects from the specific effects of chemicals on individual organisms.

In situ biomonitoring measures biological response in individual organisms. Eventually, the tests will monitor biological parameters at four levels of biological organization: cellular, tissue/organ, individual, and population.

The biomonitoring strategy utilizes a stepwise progression of sensitive tests, from simple to more complex and time-consuming; the first tests are performed on sensitive species, and provide information that enables the investigator to determine if other, more complex and expensive tests are needed. The strategy brings together regulators and scientists in a single decisionmaking process. Together, they decide whether the problem justifies additional testing, and if so, which tests should be used (6).

2. Marine/Estuarine Complex Effluent Toxicity Testing Program

General Introduction to the Marine/Estuarine Complex Effluent Toxicity Testing Program

The 1984 amendments to the Clean Water Act recommend the use of biological test methods to help prevent the "discharge of toxic materials in toxic amounts." The 1987 Water Quality Act further supports EPA's increased use of biological test methods in setting National Pollutant Discharge Elimination System (NPDES) permit limits to protect our Nation's surface waters for their designated uses. In response to the 1984 amendments, researchers at EPA's Environmental Research Laboratory in Narragansett, Rhode Island (ERL-N) developed the marine component of the Complex Effluent Toxicity Testing Program (CETTP). The program includes tests that estimate "safe" or "no effect" concentrations for toxic effluents and thus predict allowable effluent concentrations that should provide for the normal propagation of fish and other aquatic life in marine receiving waters.

U.S. EPA's Environmental Research Laboratory in Narragansett, RI.

Historically, effluent toxicity tests measured only acute or short-term (generally less than 96 hours) effects with lethality as the test endpoint. Such methods and their application are provided in "Methods for Measuring the Acute Toxicity of Effluent to Freshwater and Marine Organisms" (7). Since actual field exposures often occur at sublethal concentrations, EPA realized the need to directly test the effects of chronic (longer-term, lower concentration) exposures. The 1985 acute methods manual provides a means to protect for chronic effects by extrapolation from the acute toxicity determinations. Previously definitive chronic toxicity testing required a minimum of 30 days up to a full year to measure the actual effects of low toxicant concentrations on the entire life cycle of an organism. These full life cycle tests were developed with several fish species and with mysids but virtually no other species. Since the observed toxicity in these tests was most strongly manifested during the organisms early and most vulnerable life stages, the tests were abbreviated to include only these life stages. In an effort to produce comparable, reliable results within much shorter time periods and at reasonable costs, tests were developed that focused on several other organism's vulnerable life stages. Test endpoints were targeted to survival, growth, fecundity, and reproduction. Eventually, abbreviated marine chronic tests were developed that estimate long-term toxicity in less than 9 days. The CETTP tests evaluate the biological (toxicity) effects of complex effluents or receiving waters. The tests, therefore, include the additive effects of complex effluents on several important life functions of exposed organisms.

Researchers developed tests for six species in four phylogenetic groups under the CETTP (Table 2-1). The kelp toxicity test has just recently been developed for the CETTP. All of these tests may be used to develop permit limits and assess compliance for toxic discharges into marine/estuarine environments. The techniques in these tests are designed to minimize equipment and sample volume so that they can be conducted either on site, in a mobile laboratory, or at an offsite lab, by shipping minimal volume samples of effluents or receiving waters.

The following sections briefly describe these tests. Detailed descriptions of the test methods are provided in "Short-term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms" (8) and "User's Guide to the Conduct and Interpretation of Complex Effluent Toxicity Tests at Estuarine/Marine Sites" (9). General guidelines for conducting short-term chronic toxicity tests are presented in Table 2-2. EPA also is currently developing tests with additional marine plant and animal species from other phylogenetic groups and other geographic locations.

Red Algal (*Champia parvula*) Sexual Reproduction Test Method

This test method estimates the chronic toxicity of effluents and receiving waters on the sexual reproduction

Table 2-1. Test Species, Response Parameters, and Test Duration of the Marine/Estuarine Short-term Complex Effluent Toxicity Tests (8)

Species	Test Duration	Test Endpoint(s)
Champia parvula (Red macroalgae)	7 to 9 days total; 2-day exposure, 5- to 7-day incubation	Cystocarp production
Laminaria saccharina (Kelp)	5 to 9 days total; 2-day exposure, 3- to 7-day incubation	Sporophyte production
Arbacia punctulata (Sea urchin)	1.33 hours total; 1-hour sperm exposure, 20-minutes fertilization	Egg fertilization
Mysidopsis bahia (Mysid)	7 days total; 7-day exposure	Survival,growth, and egg production
Cyprinodon variegatus (Sheepshead minnow)	9 days total; 9-day exposure	Survival without terata
Cyprinodon variegatus (Sheepshead minnow)	7 days total; 7-day exposure	Survival and growth
Menidia beryllina (Inland silversides)	7 days total; 7-day exposure	Survival and growth

ERL-N's mobile laboratories.

of the marine macroalga, *Champia* parvula. Seaweeds had previously been considered far less sensitive to toxicity than aquatic animals, and thus not useful for toxicity testing. However, this conclusion was based on algal growth as the test endpoint. Algal reproduction tests, including those with *Champia*, can sometimes be more sensitive than tests with aquatic animals.

Unialgal stock cultures of *Champia parvula* should be maintained, so that mature plants are available year round for toxicity testing. There are three macroscopic stages in the life cycle of *Champia*, and all three look the same superficially (Figure 2-1); however, only the male and female plants are used in toxicity testing. The female production of cystocarps, which are the products of red algal sexual reproduction, is used as the endpoint.

The toxicity test with *Champia* consists of first combining male and female plants and exposing them for 48 hours, during which time fertilization should occur, and then allowing cystocarps to develop over a 5- to 7-day recovery period. Five female branches and one male branch are placed together in each test chamber, with three or four replicate chambers for each treatment. After exposure to the effluent, females are removed and placed into clean seawater. At the end of the recovery period, the number of cystocarps per plant are counted. The results are compared to a control in a series of statistical tests to calculate the "no effect" concentrations of the effluent.

Kelp (*Laminaria saccharina*) Sexual Reproduction Test Method

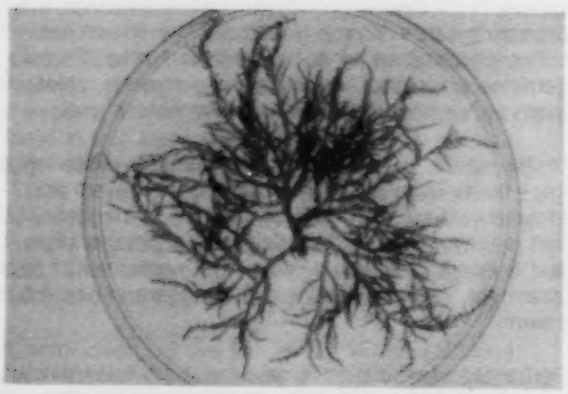
ERL-N recently developed this test method for use in the CETTP (10); therefore, the method is not included in the original test manual (8). The method of estimating the chronic toxicity of whole effluents and receiving waters on the sexual reproduction of the brown macroalga *Laminaria saccharina* is similar to that of the *Champia* test. This test is an excellent complement to the *Champia* test because, while *Champia* is found in warm waters, kelps are primarily found in cold waters throughout the west coast of North America and in New England on the east coast.

ERL-N maintains unialgal stock cultures of male and female *Laminaria* that are available for toxicity testing. Six

Table 2-2. General Guidelines for Short-term Marine Complex Effluent Toxicity Test Methods[a] (8,9)

Guideline	Description of Guidelines
Health and safety	Appropriate measures should be taken to protect against possible health hazards derived from effluents or receiving waters.
Quality assurance	All tests should be performed by aquatic toxicologists or under their supervision. Tests require high-quality test conditions to ensure confidence in test results. Quality considerations include: animal health, sample collection and handling methods, instrument condition and calibration, adequate controls and replication, accurate recordkeeping, use of reference toxicants, and test acceptability criteria.
Facilities and equipment	Tests can be conducted in an appropriately equipped stationary or mobile laboratory.
Effluent samples	Water samples should be collected just prior to the start of tests. Water samples are generally collected as 24-hour composite samples,[b] which are appropriate when the components of an effluent remain relatively constant in volume and abundance. (Note, however, that this sampling method may not adequately represent short-term peaks in toxicity. This type of toxicity must be addressed using acute tests on grab samples.) Samples of whole effluents are collected at the discharge site, while receiving waters are collected at a series of predetermined sampling stations, representative of varying degrees of impact.
Dilution water	A range of test solutions is created with varying effluent concentrations to determine the concentration of effluent that is toxic to the organism. Five different effluent concentrations and a control containing no effluent are selected for each test. Careful steps are taken to ensure that salinities in all samples are identical, and remain constant throughout the experiment. Salinity is adjusted with the aid of either artificial seawater (for some but not all species) or with hypersaline brine, made from evaporating high quality, filtered seawater. Seawater of desired salinity is created by mixing a proportion of brine with deionized water.
Endpoints for chronic toxicity tests	Endpoints in these tests include survival, growth, fecundity, and reproduction.
Statistical analysis of test results	The statistical analysis of these tests should be conducted by or with the aid of a qualified statistician. Through a series of statistical tests, two different estimates of the toxicity of an effluent are made: the NOEC (No-Observed-Effect Concentration), an estimate of the highest concentration that will not have a significant effect on the organism; and the EC (Effective Concentration), an estimate of the proportion of organisms that will show effects at any given concentration.

[a]These guidelines refer to the conduct of all tests mentioned in this section.
[b]Composite sampling is generally conducted automatically with a collection apparatus placed adjacent to a discharge. A 5-liter water sample is often collected over a 24-hour period by collecting small volumes of water at regular intervals.



Robust sample of the marine macroalga *Champia parvula*.

days prior to testing, males and females are placed separately in a standard blender and are blended to provide smaller cell clusters, filtered to remove remaining large clusters, and diluted. Males are placed in petri dishes and females on small glass slides, 100 to 150 females per slide. The cultures are maintained for 5 days in a high iron medium to allow gametogenesis to occur (Figure 2-2). If significant pregametic cells are identified after 6 days, the water in male dishes is replaced either with treatment (various effluent dilutions) or with control seawater. One slide of female parts is then placed in each of the male dishes. Exposure is for 48 hours, after which the females are removed and placed into clean seawater. After an additional 4 to 7 days, the slides are examined for sporophytes, the products of *Laminaria* sexual reproduction. The results are expressed as the number of sporophytes in test conditions compared to the number of sporophytes that develop in control seawater. The results are statistically analyzed and the "no effect" concentrations of the effluents are derived.

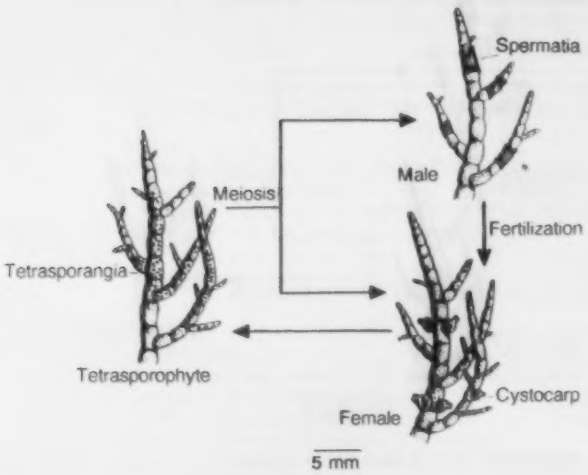


Figure 2.1. Sexual reproduction in the Red Marine Macroalga *Champia parvula (8).*

Cystocarps in a mature female *Champia parvula*.

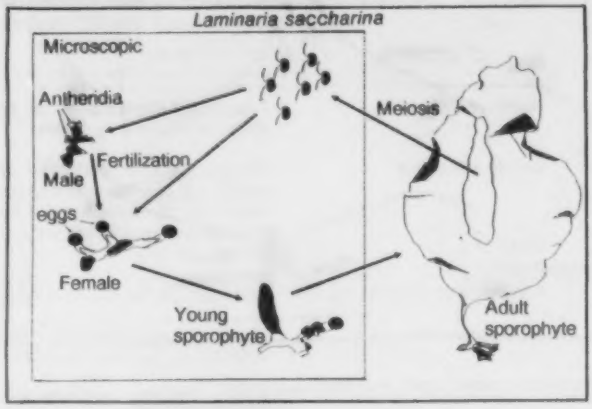
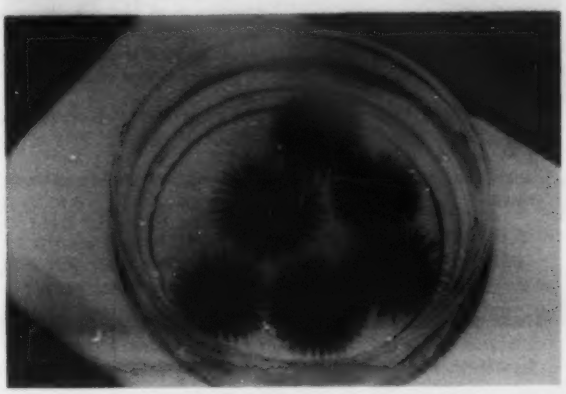


Figure 2-2. Sexual reproduction in the Kelp *Laminaria saccharina* (10).



Adult sea urchins *Arbacia punctulata*.

Sea Urchin (*Arbacia punctulata*) Fertilization Test Method

This test method, adapted from a method by Dinnel et al. (11), estimates the chronic toxicity of effluents or receiving waters to the gametes of the sea urchin (*Arbacia punctulata*). *Arbacia* adults are hardy and can be held in aquaria for long periods. This species can be induced to spawn vear round, making it especially convenient to work with. Because this test can be conducted rapidly (1 day), it can be used to quickly test a multitude of effluent types and locations.

Adult *A. punctulata* can be collected easily along the Atlantic coast or purchased from commercial suppliers. Once obtained, they are separated into male and female cultures. Male gametes are released by stimulating the organism with a 12-volt electrode. The sperm are collected and iced for not more than 1 hour. Female gametes are collected similarly and may be maintained at room temperature for several hours before use.

Urchin sperm are exposed to effluents or receiving waters for 1 hour. Then eggs are added to the sperm and allowed to incubate. After 20 minutes of incubation, the test is terminated by the addition of a preservative. The test is conducted with three or four replicates of each treatment, each containing 2,000 eggs and about 5 million sperm.

Percentage fertilization is calculated after examining a subsample from each test vial under a dissecting microscope. The test results are recorded as the percentage of eggs fertilized in test solutions compared to the percent fertilized under control conditions. The results

are used in a series of statistical tests to calculate the "no effect" concentrations of the effluent.

Mysid (*Mysidopsis bahia*) Survival, Growth, and FecundityTest Method

This test method estimates the chronic toxicity of complex effluents and receiving waters to the vulnerable period of egg development of the mysid (*Mysidopsis bahia*). Maturing juveniles are exposed to various concentrations of effluents or receiving waters in a 7-day test. Each day, new sample water is collected and prepared with appropriate effluent concentrations, salinities, and food concentrations, and maintained under appropriate environmental controls. Eight replicates of each concentration are maintained with five mysids in each treatment to ensure the statistical significance of test results. The test is completed after the mysids are exposed to the test solutions for 7 days.

At the termination of the test all mysids are transferred to clean seawater for observation. The sexes are determined and the number of immature mysids, mature males, mature females with eggs, and mature females without eggs are recorded (Figure 2-3). Dry weights are determined to calculate growth. The test results compare mysid survival, growth (weight), and fecundity (the percentage of females carrying eggs) in test solutions to mysid survival, growth, and fecundity in clean seawater controls. Statistical tests are used to calculate the "no effect" concentrations of the effluent.

Sheepshead Minnow (*Cyprinodon variegatus*) Embryo/Larval Survival and Teratogenicity Test

This test method estimates the chronic toxicity of whole effluents and receiving waters to the early developmental stage of the sheepshead minnow (*Cyprinodon variegatus*). (This method was developed at EPA Region VI Laboratory, Houston, TX.) Newly fertilized (less than 24 hours old) *C. variegatus* embryos are exposed to a range of effluent concentrations or receiving waters from shortly after fertilization, through hatching, and for 4 days into the larval period. Each day, new sample water is collected and prepared with appropriate salinity, effluent concentrations, and other environmental controls. No food is required in this test because the extremely young larvae can survive on the nutrients provided in their yolk sacs. Three or four replicates of each concentration are maintained with 10 to 15 embryos in each treatment to increase the significance of test results. The test is completed after 9 days of total exposure or 4 days of post-hatch exposure, whichever comes first.

At test termination, all live fish larvae without obvious terata are counted to determine survival in each test chamber. Survival is counted as those healthy larvae without deformity (terata) at the completion of the exposure period. There is no measurement of growth in this test. The test results are acceptable if the larvae in control cultures show survival rates greater than 80 percent. A series of statistical tests again are used to calculate "no effect" concentrations of the effluent from the results of survival and terata determinations.

Sheepshead Minnow (*Cyprinodon variegatus*) Larval Survival and Growth Test

This test method estimates the chronic toxicity of whole effluents and receiving waters to the sheepshead minnow (*Cyprinodon variegatus*). By focusing on the vulnerable early larval stage, the test estimates the impact on the entire life cycle of the fish.

In the test, newly hatched (less than 24 hours old) fish larvae are exposed to various concentrations of effluents or receiving waters for 7 days (Figure 2-4). The salinity range suitable for conducting the test is 20 to 32 parts per thousand (ppt); an appropriate salinity is selected and is held constant throughout the experiment. Each day, new sample water is collected and prepared with appropriate dilutions, salinity, dissolved oxygen (DO), and temperature. Three or four replicates of each concentration are maintained with 10 to 15 larvae in each treatment. After the new water is added each day, the larvae are fed to excess with newly hatched larval brine shrimp, *Artemia sp.* The test is completed after the minnows are exposed to the test solutions for 7 days.

At test termination, all healthy larvae are first counted to determine survival, then sacrificed, dried, and weighed to determine growth. The test results compare the larval growth or survival in test solutions to the larval growth and survival in clean seawater controls. The test results are acceptable if the larvae in the control cultures (no effluent) show survival rates greater than 80 percent and acceptable dry weights (8). A series of statistical tests are used to calculate the "no effect" concentrations of the effluent.

Inland Silverside (*Menidia beryllina*) Larval Survival and Growth Test

This test method estimates the chronic toxicity of whole effluents and receiving waters on the vulnerable larval stage of the inland silverside (*Menidia beryllina*) (Figure 2-5). This fish species is an important dietary component of many commercially harvested species including mackerel, bluefish, and striped bass. These minnows are tolerant of a large salinity range (< 5 to 32 ppt) and can therefore be useful for effluent testing in estuaries where salinities may be lower than in open ocean environments.

Test methods for this species are nearly identical to those for the sheepshead minnow survival and growth tests described previously. Seven- to 11-day-old larvae are exposed to various concentrations of effluents or receiving waters for 7 days. Results of this test are recorded as the comparison between larval growth

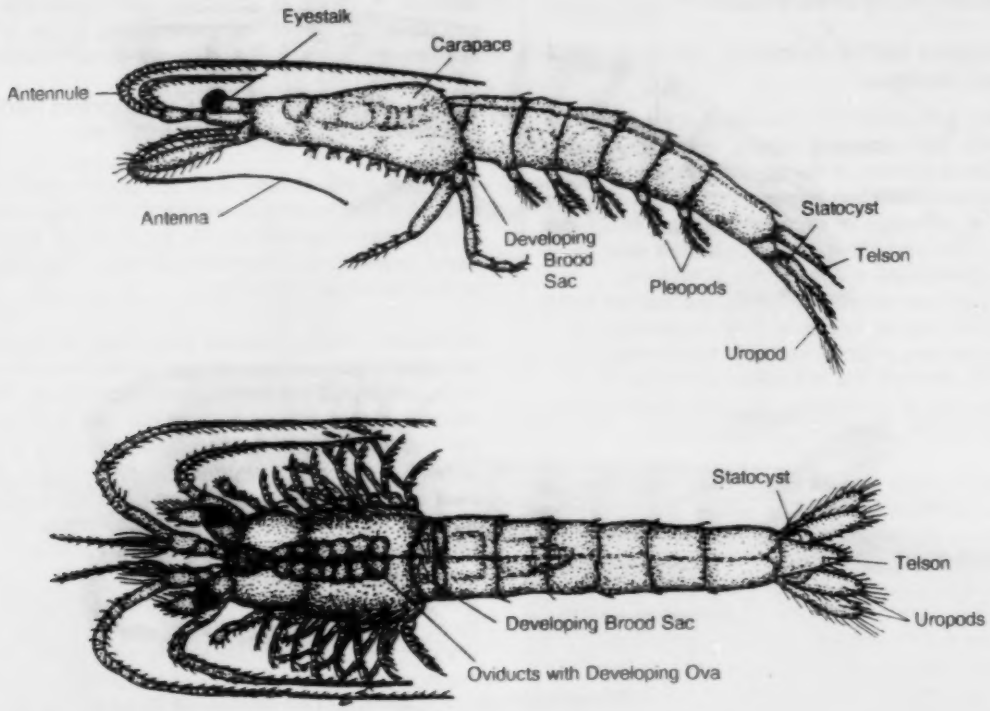


Figure 2-3. Mature female mysid Mysidopsis bahia with eggs in the brood sac (8).

Conducting the sheepshead minnow toxicity test in ERL-N's mobile laboratory.

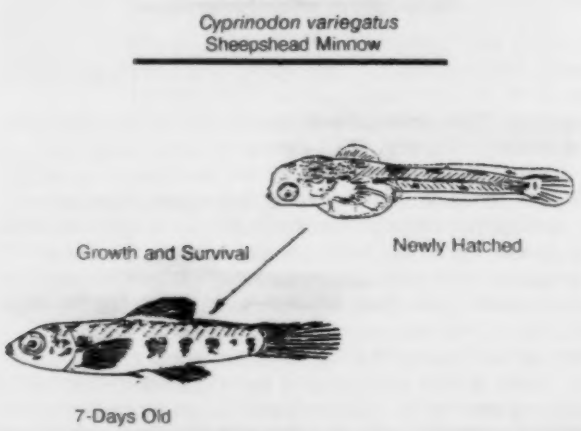


Figure 2-4. Early development of the juvenile sheepshead minnow *Cyprinodon variegatus* (8).

(weight) or survival in test solutions and larval growth and survival in clean seawater controls. Test results are acceptable if larvae in control conditions exhibit survival rates greater than 80 percent and acceptable dry weights (8). The results of survival and growth determinations are put to a series of statistical tests and the "no effect" concentrations of the effluent are derived.

Menidia beryllina
Inland Silverside

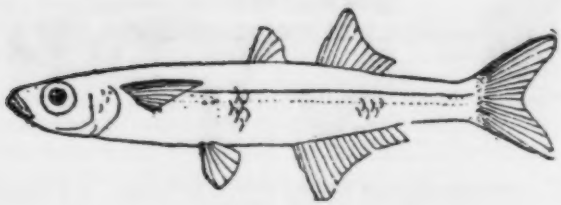


Figure 2-5. Inland silverside minnow *Menidia beryllina* (12).

A Special Case: The Toxicity of Drilling Fluids - Mysid (*Mysidopsis bahia*) Static, Acute Toxicity Test

Oil and gas drilling operations produce effluents that are frequently discharged to marine/estuarine environments (Figure 2-6). Drilling fluids, an added constituent used to lubricate the drill bit, contain primarily clays and minerals (for example barium sulfate, bentonite, lignite, and lignosulfonate), but also contain materials used as dispersants and thinners, fluid loss reducers, lubricants and emulsifiers, defoamers, biocides, corrosion inhibitors, and pH control/stability additives (13). Most commonly, the toxicity of drilling fluids is assessed by determining the acute toxicity of the fluids to mysids, *Mysidopsis bahia*, according to the method developed at EPA's Environmental Research Laboratory in Gulf Breeze, Florida (ERL-GB).

In the 96-hour test, 3- to 6-day-old mysids are exposed to various concentrations of drilling fluids. Samples are prepared by mixing the drilling fluid with seawater (1 part fluid: 9 parts seawater, v:v), allowing the sample to settle for 1 hour, and then decanting only the suspended particulate phase (SPP) for toxicity testing. If necessary, the pH is adjusted to the seawater's original alkalinity and the dissolved oxygen concentration is increased by aeration to at least 60 percent of saturation. A range-finding test should be conducted with samples of unknown toxicity to determine the range of concentrations for the definitive test. The definitive test uses mysids from a single hatch that are randomly distributed into five concentrations of the SPP plus a control. Three replicates of each concentration are maintained with 20 mysids in each. All treatments are gently aerated for the duration of the test. Mysids are fed *Artemia* at a rate of 50 *Artemia* per mysid per day. Temperature, dissolved oxygen, and pH are measured and recorded after 0, 24, 48, 72, and 96 hours.

After 96 hours of exposure, the test is terminated and the number of live mysids in each treatment is recorded. The test results are acceptable if at least 90 percent of the mysids in control conditions survived. A series of statistical tests are then used to estimate the median toxic concentration of the drilling fluid.

The toxicity of drilling fluids is not routinely assessed with the chronic toxicity tests thus far described because of the physical characteristics of the fluids which may contain materials of varying densities that separate upon settling. A special test apparatus that maintains particles in suspension could be adapted so that the CETTP tests could be used to assess potential impacts of drilling fluids.

Analysis of Test Results

The results of the CETTP tests are used to estimate the interval between the highest "no effect" concentrations of the effluents and the lowest effect concentrations of the effluents tested. The statistical methods employed are rather complex and should be handled by, or with the aid of, a statistician. U.S. EPA has prepared a complete explanation of these methods (8). The environmentally "safe" concentration of an effluent is the highest concentration of that effluent that will not have any adverse effects on the organisms in a receiving water. This "safe" or "no effect" concentration of an effluent is estimated with toxicity testing. By using statistical methods on the same set of data from a single test, two different expressions of toxicity are calculated.

The first method of statistical evaluation uses a threshold model of toxicity. The method can be used to derive the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) of the effluent. The use of NOECs and LOECs assumes both of the following premises. First, the model assumes that a true threshold concentration exists, below which there is no adverse effect to the organism and above which there is an adverse effect. In addition, the model assumes that adverse effects that are not statistically observable are not significant from a biological standpoint. If a threshold model of toxicity is accepted, NOECs will express the highest concentration of the effluent that will not adversely affect the organism tested.

The second method of statistical analysis is based on a continuous or concentration-dependent model of toxicity. Alternatively to (but not inconsistent with) the threshold model, the concentration-dependent model is used to derive Effective Concentrations (ECs). (If the endpoint being tested is mortality, EC is called LC or Lethal Concentration). By definition, any EC or LC value is an expression of *some* amount of adverse effect. For example, EC50 is the effluent concentration that would affect 50 percent of the organisms tested. Interpretation of EC values, therefore, requires the judgment of a biologist. By calculating both NOEC/LOEC and EC values

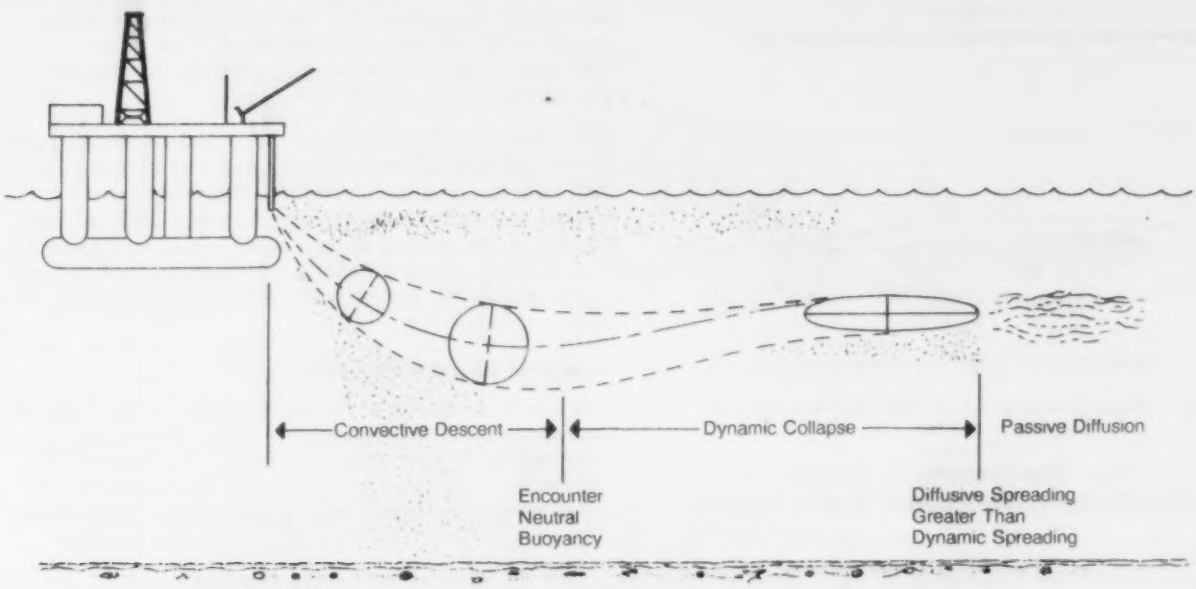


Figure 2-6. Effluent discharge plume from an oil and gas drilling operation in a marine/estuarine environment (14).

from the same results of each test, biologists can provide regulators with sound, reproducible data and a solid basis for informed decisionmaking.

Biologists should use caution in interpreting test results in cases where the site-specific effects of pH, salinity, temperature, and other natural conditions may affect the bioavailability of the toxicants in question. For instance the bioavailability of free ammonia is largely dependent on the pH of the ambient water, and thus pH can affect the apparently "safe" or "no effect" concentration of the effluent, and skew test results. An example of interactive effects between an effluent and a receiving water was encountered during the evaluation of the toxicity of the effluent discharged from a pulp and paper mill in Fernandina Beach, Florida (see Appendix A).

Field Verification of Test Methods

Validation of short-term toxicity tests is a necessary first step before they are incorporated into the environmental regulatory process. ERL-N validated these new methods first with extensive field testing. This was accomplished by comparing effluent toxicity to receiving water toxicity at comparable effluent concentrations. Scientists conducted 11 such field tests at a variety of sites with an array of discharges from chemical plants, sewage treatment plants, shipyards, and an aircraft refurbishing facility. The results of these tests indicated a good correlation between the toxicity of receiving waters and comparable effluent concentrations in the laboratory. Tests that were most sensitive to effluent concentrations in the laboratory were correspondingly most sensitive to the appropriate receiving waters in almost all cases. Case studies illustrating this correlation are provided in Appendix A.

Test Precision

Precision tests were performed within a single laboratory (intralab, ERL-N) to analyze the variability introduced by the methods and by individual species. Precision (closeness of repeated measures) is a measure of test validity. Accuracy (closeness to a standard) for toxicity tests, however, cannot be determined. The results of precision tests were compared to the precision results of similar toxicity tests for freshwater organisms; the precision of the marine tests were found to fall within the commonly accepted range (15,16).

Researchers calculated EC50 values from each toxicity test that regulators would use for setting NPDES permits. EC50 values are more useful for precision comparisons than NOECs because they are not expressed only with reference to the effluent concentrations in the test; rather, they are derived from a continuous, concentration-dependent response curve. EC50s, therefore, are discrete estimates of toxicity and can be expressed as a single number for each replicate of a given toxicity test.

Morrison et al. (17) examined EC values for five replicates of each short-term chronic test and statistically compared them for variation. Precision test results indicated that while some tests are more variable than others, the overall precision of the tests is within the commonly

accepted range for similar toxicity tests with freshwater species. The red algal (*Champia parvula*) reproduction test and the sea urchin (*Arbacia punctulata*) sperm cell test gave the most variable (yet acceptable) results, while the sheepshead minnow (*Cyprinodon variegatus*) results were most reproducible. For reliable results, EPA recommends that toxicologists use a representative of the fish, invertebrate, and plant tests when testing for toxicity. The exact testing requirements for a discharger, however, are specified on a case-by- case basis in NPDES permits.

An intralaboratory precision test of the mysid, drilling fluid acute toxicity test was conducted at the ERL-GB (18). The researchers found that the sources of variability included the condition of the sample itself, the manner in which the SPP is prepared, the condition of the test animals, and the skill and experience of the people conducting the test. They also found that the interlaboratory variability of the test conducted at 10 commercial laboratories was comparable to the variability of interlaboratory acute tests conducted with single chemicals.

Both single-lab and multilab precision tests were completed during the validation of the marine and freshwater acute toxicity tests (2). Similarly, an interlab precision test of the sheepshead minnow short-term chronic toxicity test has been completed from a total of seven tests by four participating laboratories. The labs reported very similar results indicating good interlab precision. Similar "round robin" precision tests of the remaining marine CETTP tests will be completed by spring, 1990.

Relative Sensitivity of the CETTP Tests

In adapting the CETTP from the freshwater to the marine/estuarine environment, scientists considered the greater diversity of plant and animal life in marine/estuarine waters. In an attempt to account for the extreme natural variation in biological communities, as well as constantly changing physical and chemical environments, marine/estuarine toxicity tests should encompass a wide variety of species to be representative of the environment. The complex effluents discharged can react and change and, therefore, are almost impossible to quantify or regulate through analytical chemistry alone. To take into account all of these factors, the marine CETTP uses biological toxicity tests with a variety of species types and test endpoints.

The relative sensitivity of the tests is difficult to assess because different combinations of conditions may affect each species differently. All test results, however, can be used to calculate NOEC and EC values that are used directly to set NPDES effluent permit limits. While species and endpoints differ, NOEC and EC values are directly comparable, indicating the relative sensitivity of test species. No individual test has proven consistently the most sensitive. NPDES permit limits, however, should be dictated by the results of the most sensitive species tested, i.e., the *lowest* NOEC from all the tests conducted. Therefore, the more species tested, the more protective the test results will be for the receiving waters.

The sea urchin sperm cell test is often the most sensitive and can be conducted most rapidly. Therefore, regulators are encouraged to use this test for rapid screening in conjunction with two other tests.

Incorporating Test Results into NPDES Permitting

Delegated States have the responsibility for issuing NPDES effluent discharge permits. The States are provided considerable leeway in their use of Federally approved test methods for this purpose. The "Permit Writer's Guide for Water Quality-based Permitting for Toxic Pollutants" (19) supports EPA's shift in emphasis from the solely chemical-specific approach to one integrating both the chemical-specific and whole effluent techniques as a basis for developing NPDES permits. Presently, regulators developing NPDES permits can refer to the permit writers guide, "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms" (7), and "Short-term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms" (8). EPA strongly recommends that States include both acute and chronic toxicity tests as bases for NPDES permits.

Inherent in the design of many water quality-based discharge permits is the recognition of the mixing that occurs after the effluent is released. The "design flow" of effluents into freshwater is very well understood and can be described with a variety of computer models (2). While the mixing of effluents in marine/estuarine waters is not nearly as well understood, some models are discussed in "Initial Mixing Characteristics of Municipal Ocean Discharges" (20). EPA recommends using "mixing zones" for water quality-based NPDES permits, if appropriate. Acute toxicity criteria are applied within the estimated mixing zone while chronic toxicity criteria are applied at the edge of this zone with consideration of critical mixing conditions. Treatment systems should be designed to meet the more stringent requirements (acute or chronic) in each site-specific case. While toxicity tests are water-quality based, the permits are based on allowable allocations of priority pollutants and effluent volumes, both instantaneous and averaged over various time periods. A complete description of these permitting techniques is provided in the "Permit Writer's Guide to Water Quality-based Permitting for Toxic Pollutants" (19), and case studies of their application in marine/estuarine systems are provided in Appendix A.

3. In Situ Biomonitoring

The in situ biomonitoring program at ERL-N includes toxicity tests with aquatic plants and animals as well as biological and chemical tests of bioaccumulation. During the design of the program, it was recognized that measurements of water chemistry alone (without a direct biological comparison) may only allow inferences from chemical concentrations observed in the field to actual biological effects. ERL-N is, therefore, developing techniques to assess biological effects and chemical uptake simultaneously. The goal is to provide regulators with a suite of biological and chemical tests that can be integrated for an effective assessment of the receiving water effects of complex effluents discharged into marine/estuarine environments.

Many biological methods for toxicity testing are comparable to chemical methods in terms of time and resources. A recommended strategy for toxicity evaluations, therefore, is to conduct simple biological assessments first, and then, if necessary, conduct more complex biological and chemical tests for a complete assessment. Finally, the monitoring data must be effectively incorporated into the regulatory process. Chemical-specific assessment should additionally be used when pollutant criteria are available. Most traditional biomonitoring efforts have been implemented as follow-ups to environmental enforcement measures, and thus the data collected are frequently not used to their potential. A key goal of the in situ biomonitoring program is to link scientists and regulators during the regulatory effort. This will ensure that data are gathered in concert with the needs of the regulators and are available soon enough for inclusion in the decisionmaking process.

Historical Perspective

Narragansett Bay, Rhode Island, has suffered from the coastal dumping of industrial pollutants longer than any other area in the United States. Contamination first began in 1793 when the Slater Saw Mill was built upstream of the upper bay. Over the past two centuries, as the Narragansett Bay area became more and more industrialized, textile mills, machine tool activities, jewelry and plating operations, and human waste disposal systems all contributed effluents to the bay.

This long history of effluent discharge, combined with the continual tidal flushing and riverine currents of the area, have created a contamination gradient throughout the bay. The upper reaches of Narragansett Bay (especially sediments) have been heavily impacted by pollutants while the lower portions of the bay are relatively clean. This situation has established a relatively stable contaminant gradient which has provided an ideal location for field testing marine/estuarine biomonitoring measurements.

Initial bioaccumulation studies first showed the link between contaminant concentrations in mussel tissues and proximity to industrial discharges. For regulatory purposes, however, stronger inferences had to be made directly linking the observed mussel tissue contaminant residues with actual biological toxicity. In the early 1970s, the Coastal Environmental Assessment Station (CEAS) program was developed at ERL-N as a multipurpose program to assess the relative environmental health of marine coastal waters. The CEAS program used the blue mussel *Mytilus edulis* as a biological indicator species.

The blue mussel *Mytilis edulis* has been selected for ERL-N's in situ biomonitoring program (21).

The mussels *M. edulis* and *M. californicus* are indigenous to and abundant in bays and estuaries around much of the coastline of the United States. Mussels feed by filtering particulates out of surrounding seawater, so they are excellent indicators of ambient water quality. In addition, mussels feed continuously throughout the year (without a dormant period), making them a particularly convenient species for physiological study. Finally, mussels are excellent for transplanting because large,healthy populations of animals can be found and distributed easily into field cages at various sampling sites. Another species, the hard clam *Mercenaria* sp., was explored as a test species but found not to be amenable to year-round in situ testing.

While the mussel has many advantages, its primary disadvantage for short-term toxicity testing is its reproductive habits. Species that reproduce frequently provide results that are useful for extrapolation to population level effects. Mussels spawn sporadically and infrequently and, therefore, are not useful for assessing effects on reproduction. Other species are presently under consideration as future test organisms to directly predict the population effects of toxic marine discharges through reproductive tests.

The CEAS program is an intensive effort to relate biological effects in mussels to the concentrations of contaminants observed in their tissues. An initial step in the program involved transplanting mussels on a transect along a pollution gradient in Narragansett Bay (Figure 3-1). Physiological measurements on transplanted mussels indicated a correlation between biological effect and contaminant concentration along the Narragansett Bay Transect. Subsequent testing with a variety of biological tests supports these initial findings, thus providing a strong basis for use of Narragansett Bay as a "field laboratory" for testing various biomonitoring methodologies.

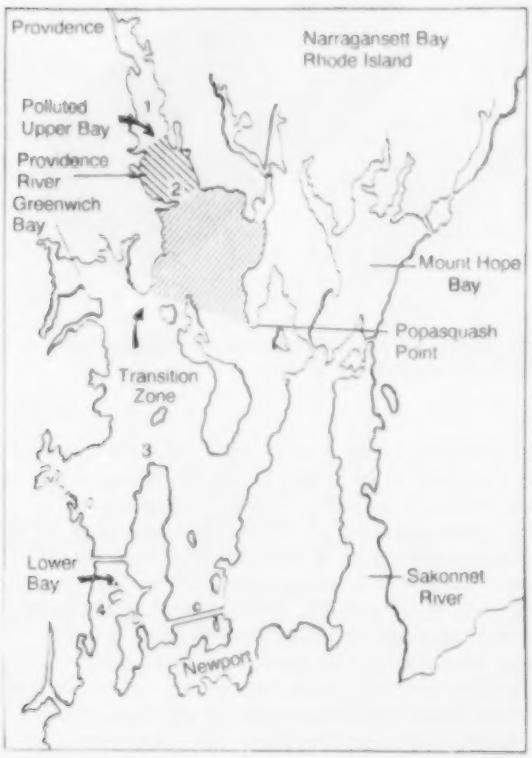


Figure 3-1. **Narragansett Bay, Rhode Island. Transect stations 1-4 (22).**

Currently, the in situ biomonitoring program at ERL-N is evaluating biological effects at various levels of biological organization. Recent results obtained from extensive field tests indicate that the biomonitoring program may provide valuable techniques to use as regulatory tools in the near future by predicting contaminant-induced perturbations in marine environments at the population level.

Biological Methods

A wide variety of biological methods have been tested at ERL-N for potential use in the biomonitoring program. The methods vary in complexity, cost, stage of development, and the level of biological organization tested (Table 3-1). Of all the biological methods explored, the scope for growth (SFG) index in *M. edulis* was found to be one of the most effective methods evaluated and now forms the primary component of ERL-N's in situ biomonitoring program. SFG (a measure of the energy budget of an animal) has been used to measure the physiological condition of mussels transplanted to polluted areas compared to those transplanted to "cleaner" conditions (23). Similarly, SFG measurements on mussels exposed to contaminated sediments in a lab exposure system have been compared favorably to those of mussels exposed to control conditions (see Section on Validation of Test Methods with Case Studies). Additionally, shell growth measurements on mussels can be used to estimate biological effects of contamination. After either field or lab exposures, chemical analyses of a subset of mussels are examined for correlation with biological effects.

Scope for Growth Procedures

Calculation of the SFG index for *M. edulis* requires the measurement of three parameters: clearance rate, assimilation efficiency, and respiration rate. Measurements are completed under standardized conditions that match exposure conditions as closely as possible, with respect to temperature and salinity. An algal food concentration of 0.5 mg/L is used because it provides sufficient energy for the organism during the tests. All SFG measurements are completed within 24 hours of collection from field or lab exposures to ensure that results are indicative of experimental conditions.A detailed description of these methods is provided by Nelson et al. (24); however, a brief summary of each procedure is provided here.

Clearance rate (CR). Mussels are placed into individual chambers through which filtered seawater, with a set concentration of algae, flows at a constant rate. After a 1-hour acclimation period, CR is determined at three hourly intervals for each mussel by measuring incoming and outgoing algal concentrations with an electronic particle counter.

Respiration rate. Respiration rates are determined by isolating each mussel in a glass respirometer vessel fitted with an oxygen electrode. The electrode is connected to

Table 3-1. Methods in Use and Under Development for In Situ Biomonitoring (8)

Method	Level of Complexity	Level of Biological Organization Tested	Description of Method
Methods Discarded			
Adenylate Energy Exchange (AEC)	High	Cellular	The amount of energy available to an organism from the pool of adenine nucleotides (ATP, ADP, and AMP) can sometimes be affected by stress. AEC is not significantly affected by contaminant concentration and has been discarded.
Blood chemistry	Medium	Cellular	Changes in the relative abundance of several normal blood components might have indicated pollution effects.
Methods Requiring Refinement			
Biomarkers including metallothionein induction, sister chromatid exchange, immunosuppression	High	Cellular	There exist several potential biomarkers or sublethal biochemical indices which might be used as early biological sentinels of pollution effects.
Histopathology	Low	Organ	Visual examination of sensitive tissues such as gills, liver, and reproductive organs may indicate structural tissue damage in response to contaminant exposure.
Tissue microbiological contamination	Medium	Organ, system	Various bacteria might be used as tags for contaminated sediments.
Demographic analysis	High	Population	Population dynamics can be inferred from the computer compilation of individual responses to contamination along a gradient.
Quantitative benthic sampling	High	Population	Community structure and contaminant effects can be determined by estimating benthic species diversity and relative abundance.
Methods that Can Be Used Immediately			
Growth, survival, and fecundity tests	Low	Individual	See Section 2 of this document.
Scope for growth (SFG)	Medium	Individual	The energy left for growth and reproduction after routine metabolic costs. SFG decreases with increased contaminant exposure. (See full explanation in this section.)
Gill respirometry	Medium	Organ	Health of animals determined by the respiration rate of excised gill tissues. (Produces results which are redundant to SFG so the test is not used.)

an oxygen meter which, in turn, is connected to a strip chart recorder. The decline in dissolved oxygen is monitored on the strip chart recorder for approximately 30 minutes. Seawater containing algae (0.5 mg/L) is pumped into the vessel during an acclimation period at a rate of 80 mL/min to ensure that food is present in the chamber and that routine metabolic rate is measured.

Assimilation efficiency. Mussels are allowed to feed overnight in the individual chambers used to measure clearance rate. The fecal material is removed from each chamber the following morning. Fecal material is dried, weighed, ashed, and reweighed to determine the ash-free dry weight to dry weight ratio. A similar procedure is completed with the cultured algae to obtain the ash-free dry weight to dry weight ratio of the food. Assimilation efficiencies are calculated for each treatment (25).

Scope for Growth Calculations

After completion of the physiological measurements, the length and volume of each mussel is measured and the tissue excised, dried, and weighed. The clearance rates

and respiration rates are standardized to the mean weight of all the mussels used in the treatment.

The weight-standardized values for each mussel are then used to calculate the SFG of each individual by substitution into the following equation:

Scope for Growth = (C x A) - (R)

where

C = energy consumed (clearance rate x surrounding food concentration x energy of food).

A = assimilation efficiency.

R = energy lost through respiration.

Shell Growth

To determine if shell growth is correlated with exposure to toxic contaminants, measurements of mussel growth are taken concurrent with field and lab exposures. The shell length of 15 individually marked mussels per cage is recorded before and after field or lab exposure. Growth is calculated as the change in shell length per unit time.

Field Exposure

Prior to a field deployment, mussels are collected with a scallop dredge 1 to 2 days before introduction into test waters. The collection site should be similar to the site of deployment with respect to temperature (< 4°C) and salinity (< 5 parts per 1000) and exhibit good water quality. The mussels are sorted to obtain a narrow size range and are distributed into polyethylene field cages (Figure 3-2). The cages are then transplanted to field sites along a gradient of contaminant concentrations, as shown for the Narragansett Bay sampling stations 1 through 4 (Figure 3-1). This technique of transplanting along a transect is the basis of in situ biomonitoring at ERL-N. After a specified deployment period, usually 7 to 30 days, the cages are retrieved and the mussels returned to the lab. A subset of mussels from each cage is used for biological testing, while the remainder of the mussels are frozen for chemical analysis.

Lab Exposure System

In addition to in situ biomonitoring, a laboratory exposure system was developed for the mussel which will provide further information for evaluating NPDES permits. Initial exposures with the system indicate that the sensitivity of the mussel to copper, a standard contaminant, is comparable to other species currently used in the CETTP.

Chemical Methods

The two methods used most commonly for determining the concentrations of specific pollutants in animal tissues

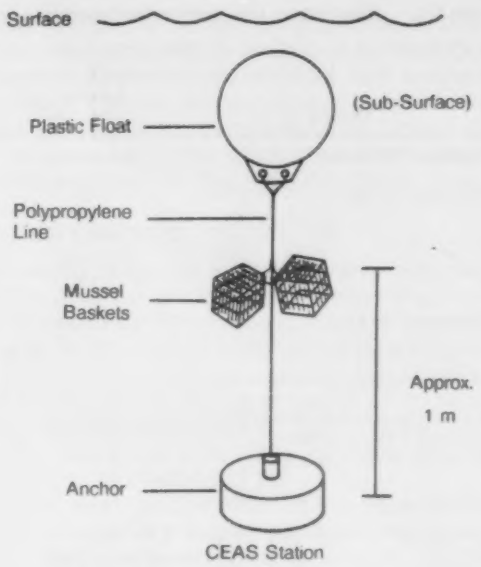


Figure 3-2. Mussel field cages used to transplant mussels along transects (22).

are atomic absorption spectrophotometry (AA) and gas chromatography/mass spectrophotometry (GC/MS). AA is used to measure trace metal concentrations, while GC/MS, together with electron capture gas chromatography, is used to determine the concentrations of volatile and semi-volatile organic chemicals like polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (26).

Validation of Test Methods with Case Studies

The sensitivity testing and validation of response parameters used within the in situ biomonitoring program have been completed through a series of case studies. One such study to field verify these methods was conducted in conjunction with the U.S. Army Corps of Engineers' Field Verification Program at Black Rock Harbor (BRH), Bridgeport, Connecticut. The approach taken was to evaluate the relationship between contaminant exposure, mussel tissue residue concentration, and subsequent biological effect. Mussels were exposed to various concentrations of contaminated sediments in the laboratory, followed by measurements of the mussels' contaminant tissue residues, SFG, and shell growth. Growth measurements as well as SFG were highly correlated with the concentration of contaminated BRH sediments to which test organisms were subjected (Figure 3-3).

A second study was initiated to determine the exposure duration period required to assess toxicity in the field. The SFG results of 1-week exposures were compared to SFG results of 1-month exposures along a contamination gradient in East Greenwich Cove (see Appendix A). Figure 3-4 shows that SFG measurements decreased

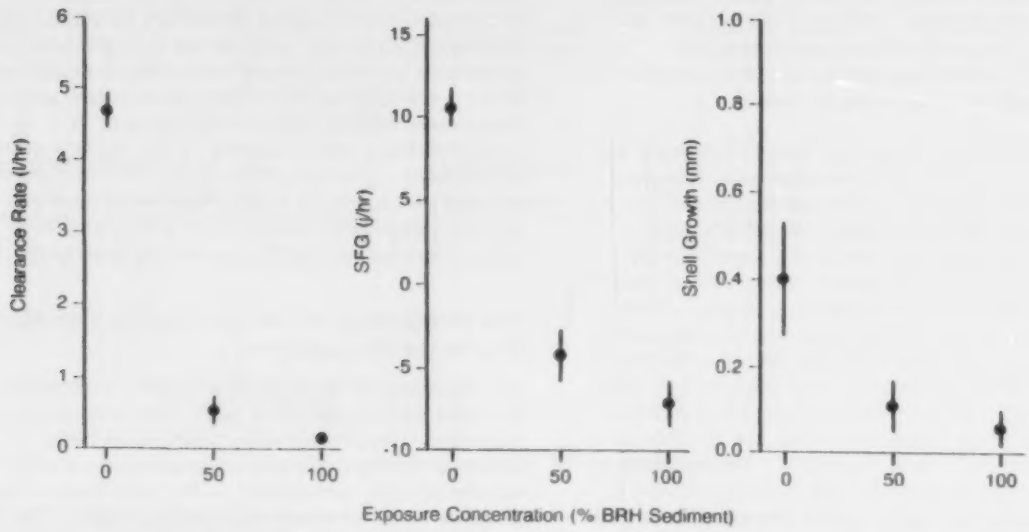


Figure 3-3. Effect of contaminated Black Rock Harbor (BRH) sediments on mussel clearance rate, scope for growth, and shell growth. Total suspended solid concentration is 10 mg/L (23).

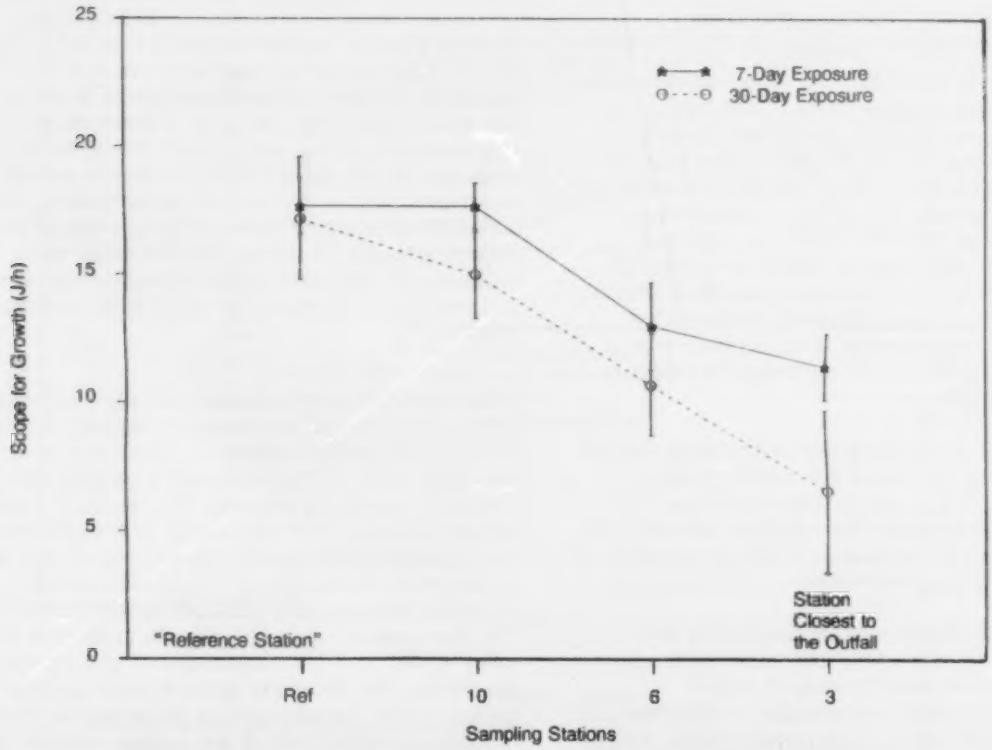


Figure 3-4. Relationship between the scope for growth of blue mussels *Mytilus edulis* after 7-day and 30-day field exposures, along a contamination gradient in East Greenwich Harbor (27).

with increasing proximity to the outfall. While 30-day exposures induced more pronounced SFG declines than 1-week exposures, the short exposure period produced significant and usable results, illustrating the utility of 7-day exposures. This study also showed that both clearance rate (CR) and respiration rate (RR) were significantly affected by exposure to toxics.

One might assume from Figure 3-3 that SFG results are redundant with simple growth measurements; however, the two endpoints can reflect different effects. SFG is a measure of all the energy available for growth, both somatic (including tissue and shell) and reproduction. Therefore, increases in shell growth reflect only a portion of the energy available to the organism. During certain periods of the year, most of the organism's energy may be allocated to reproduction and very little to shell growth. However, even during periods of slow shell growth, the organism must still maintain the physiological functions necessary for survival. Because of this, the SFG index is more reflective of the overall condition of an organism at any time of the year. In addition, the findings in East Greenwich indicate that both mussel CR and RR can be significantly affected by toxic exposure. Therefore, the SFG measurements illustrate not only the extent of the effect, but may also provide an indication of the biological mechanism behind the observed effect.

Comparisons among Various Levels of Biological Organization

Currently, research is being focused on developing tests for assessing impacts at many levels of biological organization to ensure that indicated toxicity is of real ecological significance. As a goal, tests at any level of biological organization (i.e., cellular, tissue, individual, or population) should predict the community/population effects of a discharge. The SFG index on individual mussels is *indirectly* indicative of population effects of toxic pollutants. Contaminant exposure has been shown to decrease SFG, thus lowering energy available for somatic and reproductive growth. This lowered energy may impact reproductive output and cause an effect at the population level.

The more scientists know about the mechanisms behind the observed effects, the better they will be able to interpret individual, tissue, and cellular responses and predict population responses. For example, showing that a toxicant can inhibit an organism's ability to reproduce is a way to predict the population effects of that toxicant.

Future research will concentrate on developing test methods focusing on all levels of biological organization to gain a comprehensive understanding of marine ecosystems and thus better understand contaminant-induced perturbations within these environments. Tests at the subcellular level may produce results, but those results may be difficult to interpret and extrapolate to community level effects. Changes at the community level are difficult to detect because of the often extreme natural variations that can occur in marine ecosystems. Even if detected, community level changes are extremely difficult to interpret, especially for regulatory purposes. For instance, if a change is observed in community structure or species diversity, it may only reflect natural variations within a complex and changing ecosystem, and not necessarily indicate contaminant effects. A comprehensive understanding of the biology of marine ecosystems will allow better predictions of contaminant-induced perturbations within these environments, thus providing regulators with a more solid base from which to make informed decisions concerning marine discharges.

The Integration of Biomonitoring into Marine Discharge Regulations

The implementation of marine toxicity evaluations requires the combined expertise of both marine scientists and regulators. Past monitoring efforts have been conducted without a formal means for regulators to use the monitoring data generated, so the data have rarely been effectively incorporated into decisionmaking. Because of the lack of a framework within which to interact with regulators during the implementation of toxicity evaluations, a conceptual framework for regulator/scientist interaction was developed. The framework follows EPA's principle of selecting the simplest reliable indicator of biological impact. The framework provides two opportunities for regulator/scientist interaction. In the first phase, regulators pose questions that scientists and regulators together design experiments to answer. They can select tests from the array of methods in Table 3-2. The sensitivity of the test chosen should be in accordance with the hierarchy of relative sensitivity, cost, complexity, and environmental contamination. Rapid and inexpensive biological tests with a low rate of false negative results should be used for initial site assessment, and more sophisticated biological, chemical, and physical tests should be used when indicated by toxicity.

When test methods have been selected and monitoring is in progress, the second phase of regulator/scientist interaction is invoked. A set of criteria are developed by the same team of regulators and scientists who designed the initial monitoring strategy. The decision criteria provide the team with the means to interpret the data being gathered, reevaluate the techniques and strategies employed in monitoring, and formulate decisions regarding changes and additions to the monitoring effort. The flowchart in Figure 3-5 depicts steps that might be followed during the risk assessment of a marine/estuarine discharge. The decisions made at each juncture are guided by the decision criteria developed by the regulator/scientist team at the studies initiation. Using decision criteria to ensure that proper data are gathered within appropriate time frames, scientists and regulators can beneficially interact, streamlining risk assessment and

Table 3-2. Classification of Biological, Chemical, and Physical Analyses into Four Categories that Are Subsequently Used in the Proposed Monitoring Strategy (28)

	Level 1	Level 2	Level 3	Level 4
Analysis	Survival Growth Fecundity Chemistry - inorganic water analysis	Scope for growth Gill respirometry Chemistry - organic water analysis	Population dynamics Chemistry - tissue and inorganic analyses	Chemistry - Tissue and sediment analysis Physical Bathymetry REMOTS interface Side scan sonar
Technical sophistication	Low	Intermediate-high	High	High
Time required to obtain results	Hours to days	Days to weeks	Weeks/Months	Weeks/Months

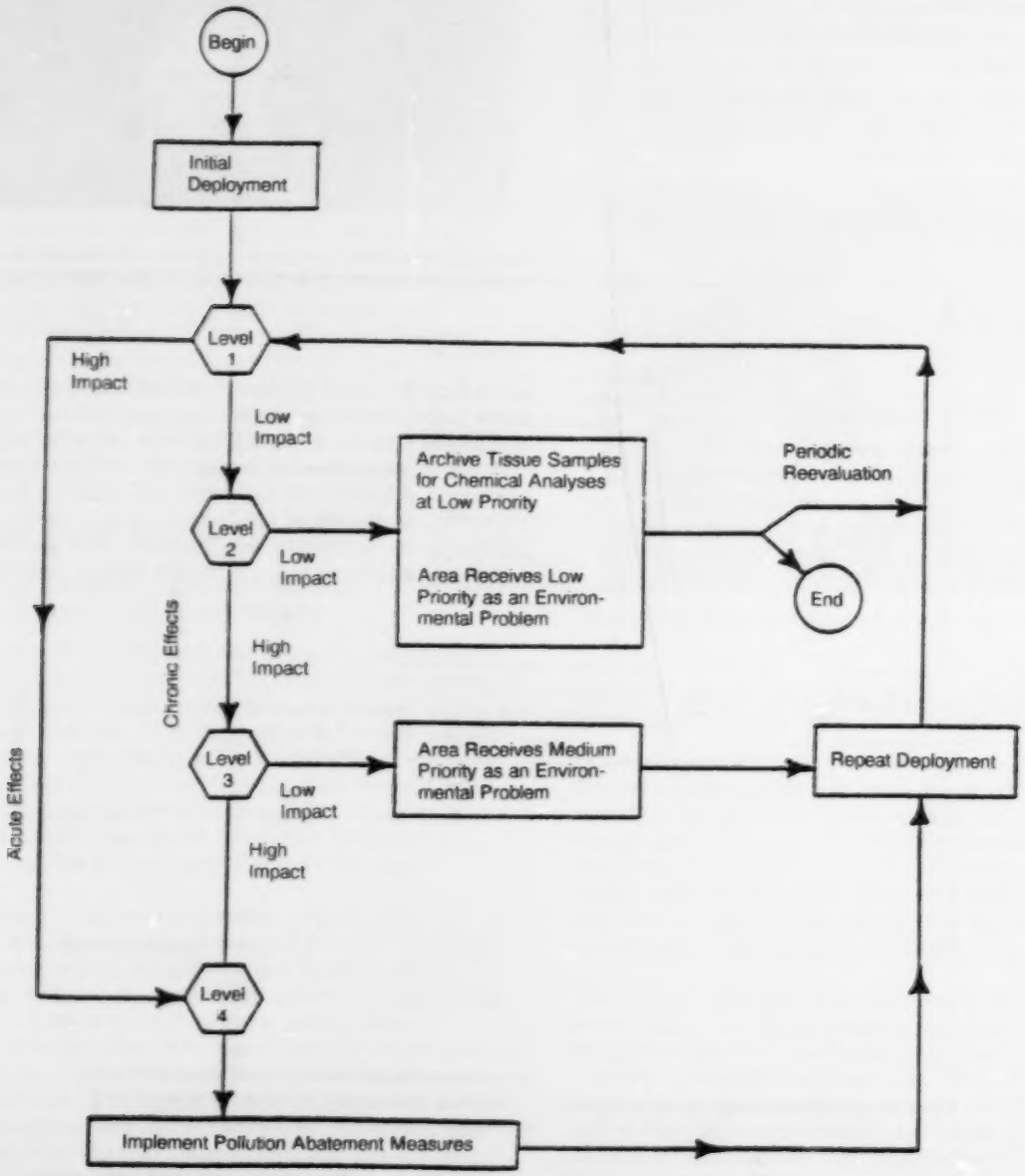


Figure 3-5. Flow chart depicting the proposed use of decision criteria in risk assessment and coastal monitoring (28).

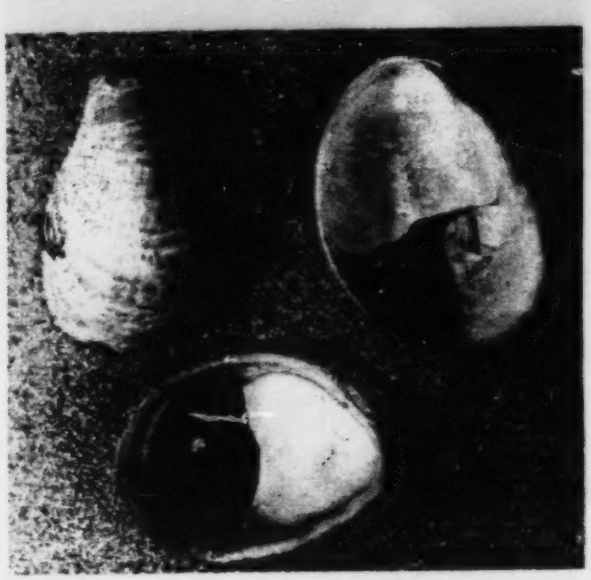
drastically reducing the cost of marine/estuarine biomonitoring. Decision criteria were effectively used during the toxicity evaluation of New Bedford Harbor, Massachusetts, described in Section 5.

Current Research

Ongoing research is currently focused on evaluating and developing several new techniques for the in situ biomonitoring program. Of particular importance is the field verification of lab results with mussels. If field verification is successful, scientists will be able to conduct reliable, rapid, and inexpensive lab studies of mussel exposure. The methods may then be suitable for incorporation into the appropriate NPDES permits. Future research will focus on:

- Tests with other species from other geographic locations - to increase the range and species representation of the tests.
- Tests with other levels of biological organization - to further demonstrate the relationship between different levels and thus provide insight into population and community level effects.

Toward these two research goals, ERL-N is currently evaluating another species, the slipper shell snail *Crepidula fornicata*, for in situ transplanting. *C. fornicata* are found on the east coast from Maine to the Caribbean and on the west coast from California to Baha. Like mussels, these filter feeders are easily transplanted and convenient for physiological studies. Unlike mussels, *C. fornicata* reproduce frequently and prolifically, which may be extremely useful for reproductive studies. The results of tests with this species may be useful for extrapolating to population level effects of toxic marine discharges.



The slipper shell snail *Crepidula fornicata* is under consideration as another species for in situ biomonitoring (21).

Summary

Upon completion of laboratory-to-field comparisons of these tests, short-term laboratory tests will be complemented by in situ assessment of receiving water conditions. In contrast to extrapolation of laboratory estimates of toxicity, in situ tests will measure the actual physiological effects of field exposure to a variety of point-source discharges with mussels, thus providing an important regulatory tool for marine toxicity evaluations.

4. Integration of Effluent and In Situ Biomonitoring Programs

Complex Effluent Toxicity

To develop NPDES permits for the control of toxic effluent discharges to marine/estuarine environments, regulators require a quick, reliable, and inexpensive procedure to estimate effluent toxicity. To accomplish this requires an estimate of both the effluent's absolute and relative toxicity. Absolute toxicity is defined as the laboratory-determined toxic concentration of the complex effluent, while relative toxicity is defined as the toxicity of the effluent after mixing with the receiving water.

Absolute toxicity is estimated with short-term CETTP tests. Relative toxicity can be estimated in two practical ways. First, relative toxicity can be estimated by sampling the receiving water at various sites and testing the samples with the same CETTP tests used to assess absolute toxicity. Alternatively, relative toxicity can be assessed with in situ biomonitoring by determining the uptake of various toxicants and the corresponding biological effects on organisms that are transplanted into the receiving water.

Receiving Water Toxicity Estimates

Use of Dye Studies to Estimate Effluent Concentrations in Receiving Waters

While laboratory toxicity tests on various concentrations of effluent provide an estimate of "end-of-pipe" toxicity of a discharge, for regulatory purposes it is essential that the toxic concentrations of complex effluents determined in the laboratory correlate with the actual effluent concentrations observed in the field. The concentration of effluent in receiving waters at field sites, however, is difficult to predict due to the complex mixing and dispersion of the effluent after it leaves the pipe.

To estimate actual receiving water concentrations of effluents, dye studies are performed. Prior to sampling any receiving water, the effluent is saturated with rhodamine WT dye by continually adding the dye to the effluent for 3 days or until an initial steady state is reached. Subsequently, when sampling occurs at various receiving water sites, the dye should be in equilibrium with the effluent. The concentration of dye in the effluent can be measured with fluorometry (fluorescence), and used along with the dye infusion rate and the effluent discharge rate to calculate the concentration of the effluent at each sampling site. In relatively stable environments, dye studies can be a powerful tool for comparing known, effluent concentrations in the lab with effluent concentrations at various field sites. In certain site-specific conditions, however, dye studies can be drastically altered by local environmental conditions. For example, biologists should use caution when interpreting the results of a dye study conducted in shallow or wind-driven estuaries.

Researcher collecting a water sample during a dye study.

CETTP Tests of Receiving Waters (Relative Toxicity Estimates)

Once dye studies have been conducted and the effluent concentrations at field sites have been estimated, short-term chronic toxicity tests are conducted with receiving waters. Sampling sites vary in their proximity to the discharge site. Toxicity tests on receiving waters are conducted with the same set of test organisms used with complex effluent samples alone, and the results are again used to estimate the toxic concentration of the effluent.

ERL-N found that the toxic concentration of the effluent measured in the lab is almost always the same as the toxic concentration of effluent in parallel receiving waters. The high correlation between lab and field results enables regulators to incorporate these tests into NPDES permits with a high degree of confidence. While the tests are attractive because they can be conducted rapidly, in situ biomonitoring might provide supplemental information on

longer-term effects. One of the longer-term in situ chronic toxicity tests is described in the following paragraphs.

Use of In Situ Biomonitoring with Mussels to Complement CETTP Tests

The efficacy of marine/estuarine complex effluent tests in rapidly estimating an effluent's toxicity makes them an important regulatory tool. However, there is concern that the very speed of the short-term tests may compromise their long-term accuracy. This becomes increasingly important during implementation because Regions and States exhibit a wide latitude in their monitoring requirements. For example, if all samples are collected during 1 intensive week, but the effluent discharge continues throughout the year, this "grab" sample technique may not account for:

- Temperature and seasonal effects, and noncontinuous discharge rates from effluent tanks or reservoirs.
- Differences in effluent components over time.

To ensure that short-term toxicity evaluations are consistent with long-term trends, it is recommended that long-term in situ biomonitoring supplement the current CETTP tests in certain site-specific discharge situations. Through periodic deployments throughout the year, mussels can be used to monitor long-term trends (Section 3). In situ toxicity tests with mussels may eventually complement CETTP tests, either by monitoring a specific discharge or as a general monitor of receiving water health.

Integrated Approach for Toxic Discharge Control

The integrated water quality-based approach of using in situ monitoring with existing CETTP methods for toxic discharge control may be a powerful tool for regulators. This approach will determine ambient water quality by measuring SFG on mussels and thereby assess the toxicity of an effluent (Section 3). The first step in this approach will be to compare SFG response of mussels from the laboratory exposure system with field-exposed mussels, over 7 days. A second step will include a comparison of the sensitivity of 7-day lab exposure with mussels to the sensitivity of short-term CETTP tests.

Point-Source Evaluations and Large-Scale Coastal Monitoring

The integrated water quality based approach, combining toxicity testing of effluents and receiving waters with ambient toxicity testing, could be further incorporated into large-scale coastal monitoring systems. The National Oceanic and Atmospheric Administration's (NOAA) Mussel Watch Program already uses in situ biomonitoring, with mussels as regional indicators of marine ecosystem health. This monitoring program periodically collects mussels at about 150 coastal and estuarine sampling sites, and measures mussel tissue residues of tracer contaminants including PCBs, PAHs, and several heavy metals (29). High tissue residues might indicate potential toxicity problems within a region. In this way, Mussel Watch can identify problem areas that require additional testing to pinpoint discharge sites or upstream runoff problems. EPA's new Environmental Monitoring and Assessment Program (EMAP) will be especially important in addressing ecological status and trends. EMAP will be phased into implementation in 1992 and will integrate the existing ecological monitoring programs concerning the Nation's forest, wetland, near-coastal, inland surface water, and agricultural areas. The near-coastal component of EMAP might be an extremely useful tool for integrating the results of localized marine toxicity evaluations and large-scale marine monitoring efforts.

Combining the integrated approach with large-scale near-coastal monitoring programs may allow the tracking of pollutants from "end-of-pipe" through receiving waters to estuaries and open bays. This integration has the potential to vastly increase the ability to assess the results of existing pollution problems. Baseline data of this kind also will help to assess the field effectiveness of remediation efforts.

Hierarchical Framework for Biomonitoring Tests

To further increase the efficiency of marine/estuarine biomonitoring, it is proposed that all biomonitoring techniques be placed into a hierarchical framework of test complexity. Each test will, therefore, be incorporated into toxicity evaluation, according to the severity of the problem. Section 3 describes the hierarchy of existing tests, and the framework within which researchers and managers can interact to best use their limited resources to select from the array of tests and control pollution of our Nation's estuaries and oceans.

The most effective approach to controlling toxic discharges is for scientists and regulators to work together closely to design appropriate control strategies. Biological tests that both rapidly estimate toxicity and monitor the long-term trends of point-source discharges can be used in a framework within which managers and regulators can evaluate the data and make decisions rapidly. This will ensure that scientists provide the answers most needed by regulators, in a timely fashion. In addition, a tandem approach for localized or point-source toxic control can be integrated into large-scale coastal monitoring programs. The integration of these monitoring programs could provide a dynamic picture of the local effects of toxic discharges and subsequent remediation measures, as well as the larger-scale effects in near-coastal waters.

5. Application of the Biomonitoring Strategy: The New Bedford Harbor Pilot Dredging Project

Introduction

New Bedford Harbor is located between the city of New Bedford and the town of Fairhaven in Buzzards Bay, Massachusetts (see Figure 5-1). Since the 1940s, electronics and manufacturing companies in the area have discharged PCB-laden effluent into the Acushnet River and the harbor. High PCB concentrations in the river and upper harbor sediments were first documented in 1974 (30). Over the past 15 years, nearly 18,000 acres of PCB- and heavy metals-contaminated sediment have been described, with PCB concentrations as high as 1,000 parts per million (ppm) in some areas of the harbor (31). In 1982, the site was added to EPA's National Priorities List of hazardous waste sites slated for cleanup under the Superfund law.

EPA's 1984 feasibility study proposed several alternatives for the harbor cleanup. One of the alternatives included dredging contaminated sediments and disposing of them in either a land-based, confined disposal facility (CDF), or an aquatic, confined aquatic disposal (CAD) area. Federal, State, and local officials, as well as the public, expressed concern over these alternatives. Many thought that the sediments stirred up during the dredging would affect the plants and animals that inhabit the harbor's waters. Others cited potential pollution problems from contaminated water (leachate) leaking from the proposed disposal site (32). To answer these concerns, EPA decided to pre-test the dredging and disposal options. Working with the U.S. Army Corps of Engineers (COE), EPA designed a pilot study to determine the best remediation option for the Superfund site.

Dredging Project Description

The COE examined and compared the effects of three hydraulic dredges and two disposal methods during this project. The dredges were selected based on their ability to remove sediment with the least amount of resuspension and to operate in shallow water. The latter was important because the dredging area was only 4 to 5 feet deep at high tide. The first dredge was a cutterhead dredge; the second, a horizontal auger dredge called a "mudcat"; and the third, a specially constructed dredge called a "matchbox" (34). The two disposal methods investigated were: 1) a confined disposal facility (CDF), which consisted of a containment dike partially in water and partially on land; and 2) a confined aquatic disposal cell (CAD), which was an in situ underwater disposal method.

The COE dredged two areas in the pilot study cove (Figure 5-2). Contaminated sediments from dredge area 1 were disposed of into the CDF (Figure 5-3). The underlying "clean" sediment was also dredged to the CDF to "cap" the contaminated material. The resulting depression in area 1 became the CAD cell (Figure 5-4). The dredged material from area 2 then was pumped into the CAD cell, and a second "clean" layer of underlying sediment from area 2 was used to cap the underwater disposal area.

Monitoring Strategy

The pilot project was an opportunity to test the biomonitoring research and management strategy described in Section 3. Before the COE began any operations, baseline physical, chemical, and biological measurements were completed. The biological measurements were used to assess the effects of existing water quality on plant and animal survival, growth, and reproduction. These tests served as a benchmark against which increased contamination and/or toxicity associated with the operational phases of the study were compared.

The determination of whether the operation caused an unacceptable effect was complicated by the fact that State and Federal water quality standards for PCBs and certain heavy metals were already exceeded under preoperational baseline conditions. In addition, the U.S. Food and Drug Administration (FDA) action level for PCBs in seafood was exceeded before dredging started.

In order to assess the effects of the dredging project, therefore, it was necessary to develop a set of site-specific numerical values, called Decision Criteria (Table 5-1). These criteria were compiled in a Decision Criteria Document (8), and were established for measured physical, chemical, and biological parameters, based on the preoperational data. During the dredging and disposal operations, data on the same physical, chemical, and biological parameters were collected and compared to the Decision Criteria values. A Decision Criteria Committee,

comprised of representatives from ERL-N, EPA Region I, the COE, and the State of Massachusetts, evaluated the data on a daily basis. If the Decision Criteria values were exceeded, the committee could require engineering corrections to the dredging operation before work was resumed. In this way, the marine environment and its resources were protected during the pilot study activities.

Water Quality Monitoring

Four stations were selected for water quality monitoring in NBH, in addition to a reference station located in Buzzards Bay (Figure 5-5). Station NBH-1 was located north of the dredge site; Station NBH-7 was adjacent to the cove where dredging occurred; Station NBH-2 was at the Coggeshall St. Bridge, the transition point between

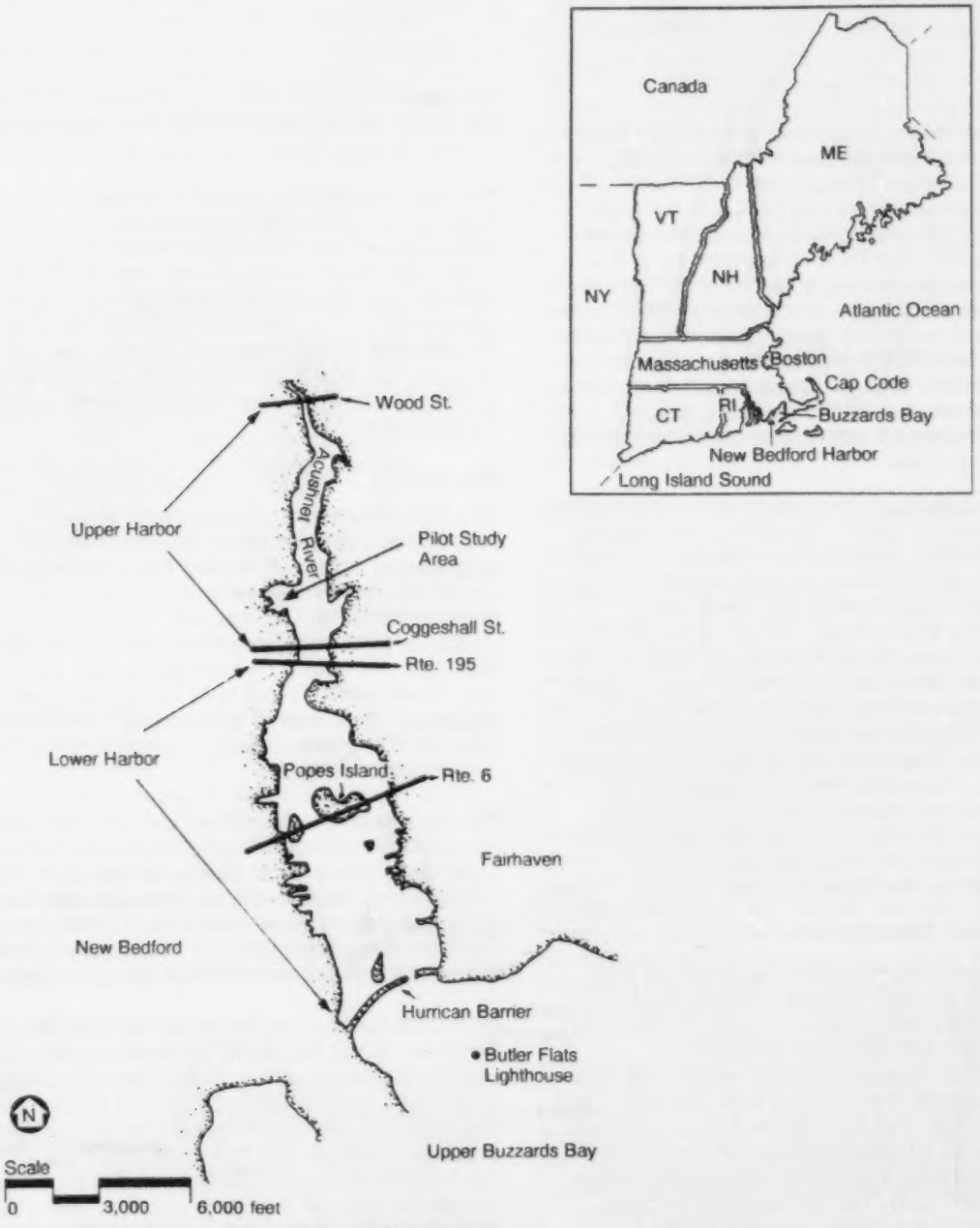


Figure 5-1. New Bedford Harbor, in Buzzards Bay, Massachusetts (33).

Aerial view of the New Bedford Harbor confined disposal facility (CDF). The silt curtain surrounding the study area extends from the far edge of the CDF dike.

the more severely polluted upper harbor and the lower harbor; and Station NBH-4 was at the hurricane barrier, the transition point between NBH proper and Buzzards Bay. The reference station for all water quality monitoring was NBH-5, located at West Island in Buzzards Bay. Mussels were deployed at stations NBH-2, NBH-3, NBH-4, and NBH-5. Station NBH-6 was used as a mussel reference station for the first deployment only; subsequent deployments utilized NBH-5 to facilitate mussel collections.

Seawater was collected separately for the ebb and flood tide at each NBH station. This method allowed estimation of net transport of PCBs and metals over time, especially at NBH-2. Each water sample was analyzed for various physical (total suspended solids) and chemical constituents (PCBs, copper, cadmium, and lead). In addition, a suite of biological tests were conducted to assess acute effects (in fish, mysids, mussels, sea urchin sperm cells, and a red alga) and chronic effects (fish growth, mysid growth and reproduction, mussel scope for growth, algal reproduction). A typical 7-day monitoring cycle is presented in Figure 5-6.

Once the operational phases of the Pilot Project began, water samples were collected in a manner similar to that of the preoperational phase. However, during the operational phase the ebb samples were returned to ERL-N immediately and chemical analyses and acute biological tests were completed overnight. These results were transmitted to the Decision Criteria Committee prior to the start of that day's dredging. The committee would then assess any adverse environmental impacts and make any necessary adjustments to the operation. This "real-time" monitoring allowed the project managers to make timely decisions based on actual data, thus ensuring the best degree of environmental protection possible.

Summary of Results - Operational Phase

Physical measurements. The temperature and salinity data were similar among the NBH stations during this study. Within the harbor, temperature decreased slightly with depth, and salinity increased minimally with depth, reflecting input from the Acushnet River at the head of the estuary. Total suspended sediment (TSS) measurements at the sampling stations indicated that the dredging operation resulted in little or no increase in TSS concentrations over background. On one occasion an increase in TSS concentration occurred at NBH-2. This was caused by the opening of the silt screen around the operation site which normally worked to contain suspended particles within the study area. This resulted in the discharge of a plume from the construction site. The problem was quickly rectified and never recurred.

Water Chemistry. The PCB concentrations in NBH exceeded the Decision Criteria values on only four occasions (at NBH-2) during the operational phase of the project. Three of these instances could be directly attributed to a specific operational event. In all cases, modification of the operation resulted in lowered PCB concentrations in the water the following day. The only other exceedence was caused by a meteorological event: 50 mph winds at low tide caused resuspension of contaminated sediment from the upper harbor. At no time did the concentrations of copper, cadmium, and lead exceed the Decision Criteria values as a result of any operational event.

Mussel Chemistry. Mussels deployed in NBH showed a distinct spatial and temporal pattern with respect to PCB uptake. The PCB tissue residues from mussels deployed before dredging indicated that mussels located in the upper harbor exhibited the highest PCB concentrations in their tissues; the concentration decreased moving down the harbor (Figure 5-7). This pattern was consistent regardless of length of field exposure. The data indicated that PCB tissue residue concentrations also increased with length of exposure. These preoperational data demonstrated the bioavailability of PCBs in NBH under background conditions. Analysis of mussel tissues exposed during operational phases of the project showed the same pattern as the preoperational exposures. Therefore, no additional increase in PCB bioavailability could be attributed to the dredging operation.

Biological Tests. The sea urchin (*Arbacia punctulata*) sperm cell test and the growth and survival tests with the sheepshead minnow (*Cyprinodon variegatus*) indicated no toxicity at any of the stations. The preoperational red algae (*Champia parvula*) reproductive tests proved inconclusive because of problems with stock cultures. Toxicity to this species was observed on several occasions during operation. However, it was not correlated with measured PCB concentrations and may have been caused by elevated copper concentrations in the water from a source other than the dredging

operation, as the red macroalgae are particularly sensitive to copper. Growth and reproduction in the mysid (*Mysidopsis bahia*) indicated no effects as a result of the dredging operation.

Mussel SFG and shell growth values from the first preoperational deployment demonstrated a significant inverse relationship with PCB tissue residue concentrations in NBH (Figures 5-7 and 5-8). In addition, SFG values on day 7 were reflective of results obtained on day 28. This pattern was not evident during a second deployment and was attributed to the fact that mussels were at the peak of the gametogenic cycle at this time. During gametogenesis, the mussels would expend extra amounts of energy and the SFG measurement would be affected. The SFG values of mussels collected during the operational phase of the project never exceeded the Decision Criteria values; therefore, no adverse impact could be attributed to the operation.

Summary

The New Bedford Harbor Pilot Dredging Project was a unique opportunity to use biomonitoring techniques, ranging from the CETTP tests to in situ exposures, to evaluate a potentially environmentally damaging dredging operation on a "real-time" basis. A set of site-specific criteria was developed and utilized by environmental managers to assess, on a day-to-day basis, the effects of this dredging operation on water quality in NBH. The results indicated that the dredging operation had a minimal effect on existing water quality. On those occasions when elevated PCB concentrations were detected, they could be directly attributed to a specific operational procedure and modified before environmental

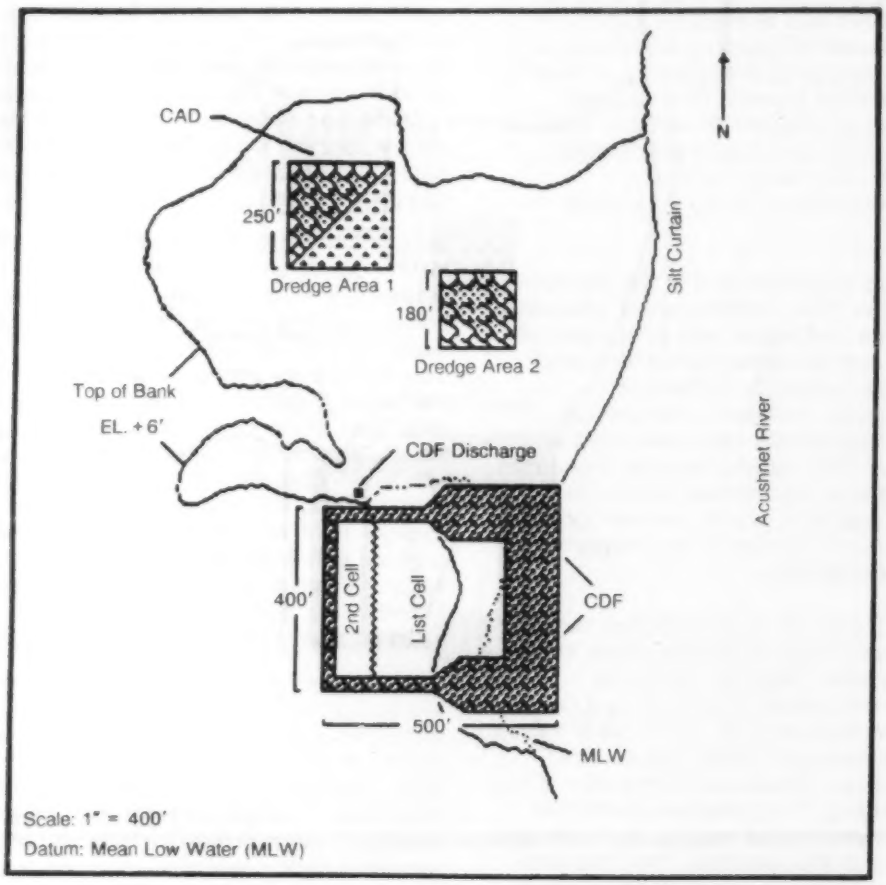


Figure 5-2. Dredging areas and disposal facilities at New Bedford Harbor pilot study area (35).

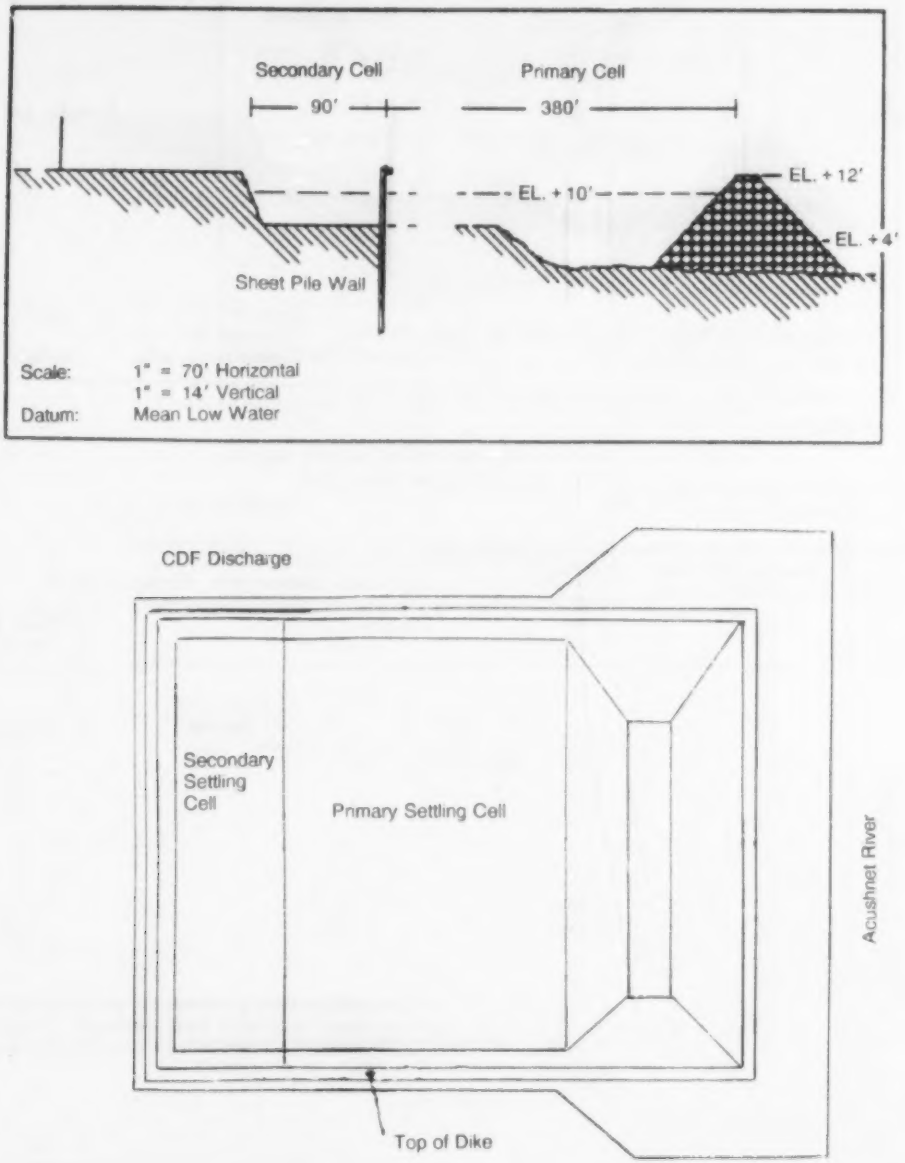


Figure 5-3. Cross section and top view of confined disposal facility (CDF), showing the two settling cells (35,34).

damage was done. The result was that no acute or chronic biological effects were observed as a result of this operation. While the approach to real-time monitoring can be labor intensive, its application is recommended when an operation, such as dredging a Superfund site, can potentially have severe environmental impacts. The Corps of Engineers is presently reviewing the data gathered during the operation, and will decide on the best dredging and disposal option for the more contaminated portion of New Bedford Harbor

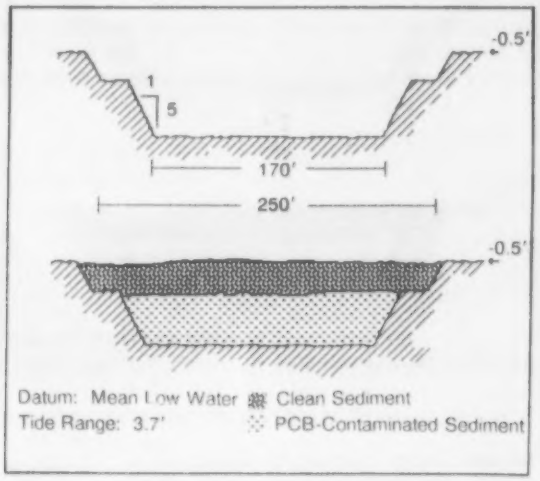


Figure 5-4. Cross section of confined aquatic disposal (CAD) cell, unfilled and filled (35).

Table 5-1. Numerical Decision Criteria for New Bedford Harbor (36)

Endpoint	Time to Obtain First Value	New Bedford Harbor Station Location	
		Coggeshall St. Bridge (NBH-2)	Hurricane Barrier (NBH-4)
Water Chemistry			
		Ebb (µg/L)	*Net Transport (kg)*
PCB (total)	24 hr	*1.4*	*0.71/cycle*
Cd	24 hr	*9.3**	
Pb	24 hr	*7.2*	
Cu	24 hr	*13.0*	
Mussel PCB Tissue Residues (µg/g)			
7-day exposure	10 days		80
28-day exposure	30 days		160
Biological Responses			
Acute (% Survival)		*Ebb*	
Fish Mysids Mussels Sea Urchins (% fert.) Red Algae	All tests monitored daily	Mortality >20% of control value for two species or >50% for one species	
Chronic Effects			Ebb**
Fish (dry wt., mg)	8 days		20% (40%)
Mysids (dry wt., mg)	8 days		20% (40%)
(reproduction)			50% (100%)
Mussels:			
scope for growth (J/h)	8 days		7.5 J/h (15 J/h)
shell growth (mm)	28 days		50% (100%)
Sea Urchin (% fert.)	1 day		25% (50%)
Red Algae (# cystocarps)	8 days		50% (100%)

*U.S. EPA water quality criterion; criteria-continuous concentration.

**This value represents a statistically and biologically significant reduction from control values. A significant reduction in two endpoints, or a twofold reduction in one endpoint (in parentheses), requires that the decision criteria committee evaluater the monitoring dataand other information related to the operation prior to the resumption of operations on the next day.

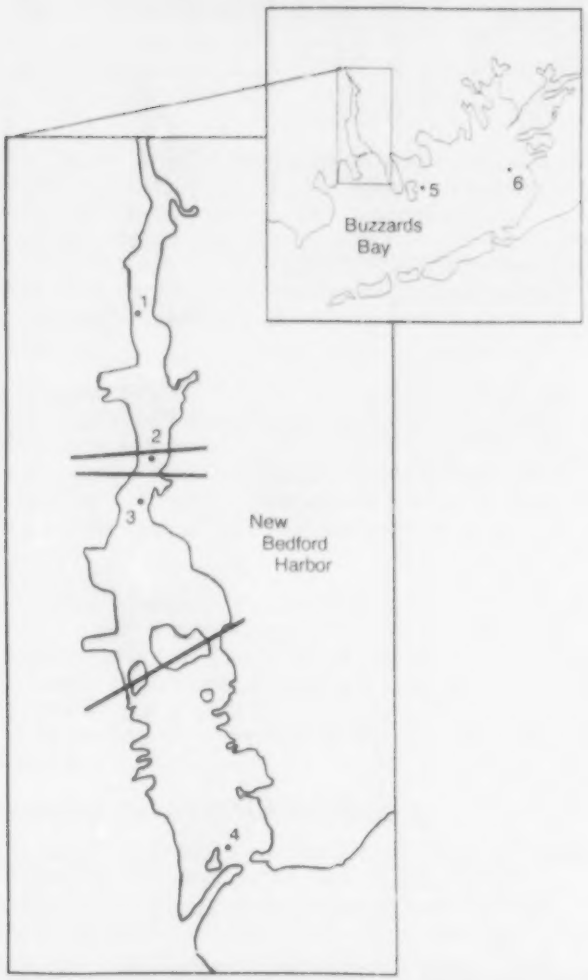


Figure 5-5. **New Bedford Harbor sampling station locations for biological, physical, and chemical tests during preoperational and operational monitoring (37).**

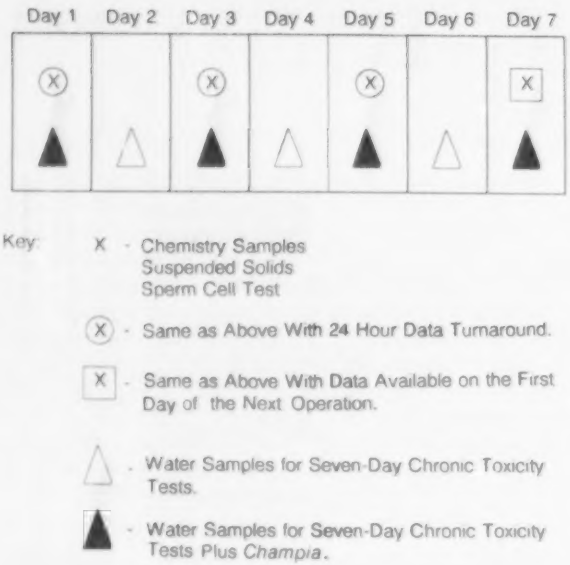

Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Key:
X - Chemistry Samples
Suspended Solids
Sperm Cell Test
(X) - Same as Above With 24 Hour Data Turnaround.
X - Same as Above With Data Available on the First Day of the Next Operation.
- Water Samples for Seven-Day Chronic Toxicity Tests.
- Water Samples for Seven-Day Chronic Toxicity Tests Plus *Champia*.

Figure 5-6. **Sampling scheme for a generic 7-day sampling period during the New Bedford Harbor pilot Study (38)**

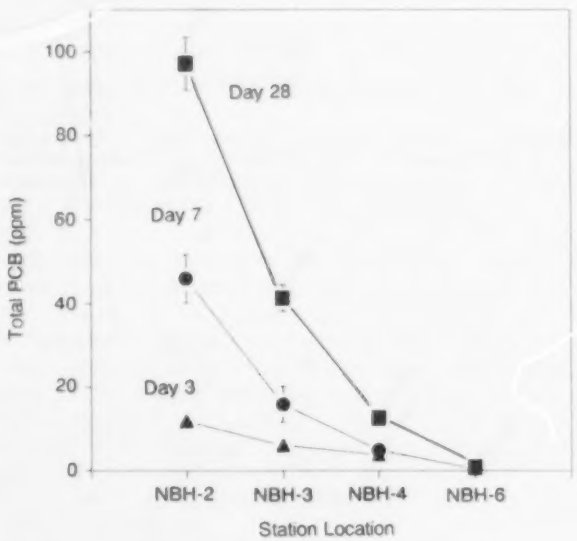


Figure 5-7. **PCB concentrations in mussel tissues over time versus total PCB concentration as determined from receiving water analysis (39).**

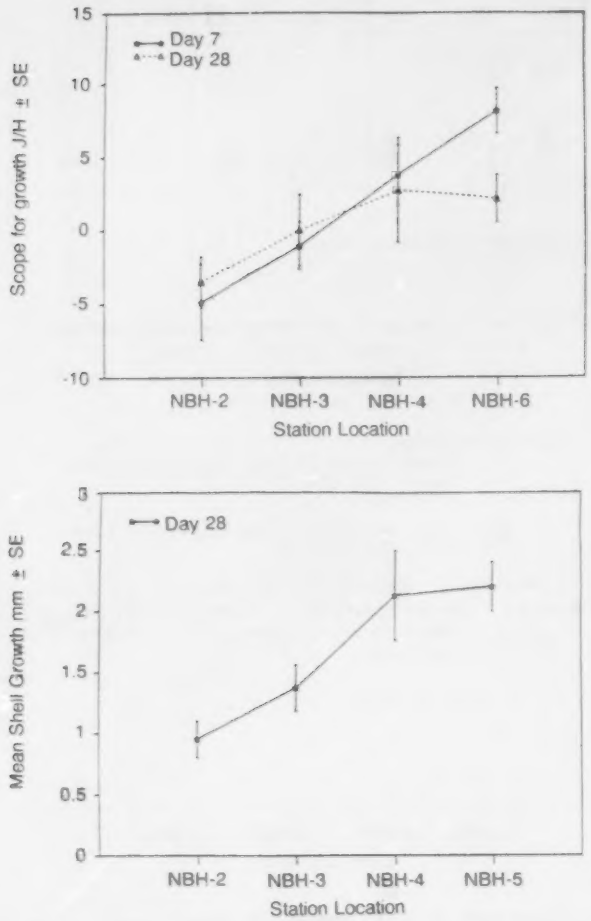


Figure 5-8. **Mussel scope for growth (SFG) in New Bedford Harbor at 7 and 28 days, mussel shell growth at 28 days. Note that 7-day SFG tests are predictive of the 28-day trends in SFG and shell growth (37).**

6. Conclusions and Future Directions

The Clean Water Act emphasizes the use of biological monitoring as the basis for maintaining national water quality standards. Inherent in the process of environmental protection is the definition of the baseline health of the ecosystem under scrutiny. Only when such a baseline is established can trends be identified and subsequently correlated with natural or anthropogenic causes.

A Complex Effluent Toxicity Testing Program has been well established for freshwater systems, but modifications were necessary to adapt the methods to marine/estuarine environments. EPA has designed state-of-the-art marine toxicity test methods to serve as the marine counterpart to the freshwater program, as presented in previous sections.

In developing the toxicity test methods, two distinct, yet related, goals were defined: 1) to develop tests that rapidly estimate the toxicity of effluent discharges so that regulatory actions can proceed and 2) to enhance the comprehensive understanding of ecological systems and trends and thereby contribute to long-term environmental protection.

Complex Effluent Toxicity Testing

The Marine/Estuarine Complex Effluent Toxicity Testing Program is currently used to help develop NPDES permits. Test species include sheepshead minnows; inland silversides; mysids; a sea urchin; and two macroalgal species, *Champia* and *Laminaria*. These species were selected for the following reasons:

- Laboratory cultures can be easily cultivated.
- Gametes or larvae can be obtained year round for shipping to onsite locations.
- Gametes or larvae require small volumes of exposure water.
- Tests are inexpensive to conduct and can be easily taught.

Future Research and Refinements to Complex Effluent Toxicity Testing Program

To increase the range and accuracy of the marine/estuarine CETTP, new species from other phylogenetic groups and other geographic areas will be added to the program (specifically, from the west coast). In addition, future research will focus on special environments, including brackish (low salinity) and tropical waters. Biomonitoring techniques for brackish water would be useful for discharges at estuary headwaters including many areas within the Chesapeake Bay and the Gulf of Mexico. Tropical species are needed to test effluent discharges in the Hawaiian Islands, Virgin Islands, Puerto Rico, Guam, and other U.S. protectorates. This is particularly important because most States do not allow the introduction of nonindigenous species.

In Situ Biomonitoring

ERL-N has developed in situ biomonitoring methods to enhance and augment the short-term test methods and monitor long-term trends of marine discharges. The in situ program relies on mussels transplanted along dilution gradients that start near the discharge site and end in clean water. By correlating the contaminant levels in the mussel tissues and subsequent biological effects, over time, scientists can determine environmental trends near to and at various distances from a discharge site. Additionally, a newly developed laboratory exposure system can help to field verify the laboratory CETTP tests.

Future Research and Refinements for In Situ Biomonitoring

The most successful biomonitoring tests to date have been conducted at the individual organism level. At higher levels of biological organization, the complexities of biological systems increase tremendously. Monitoring at the cellular and subcellular levels may simplify marine monitoring because, rather than predicting toxic effects on only one species, indicators at the cellular and subcellular levels might predict effects across many species. However, before this can be done, relationships among cellular, tissue, individual, and population effects must be understood, to justify extrapolation from one level to the next and to explain the mechanisms behind whole organism effects. Several promising techniques are currently being investigated to expand the marine in situ biomonitoring program.

For example, the slipper shell snail *Crepidula fornicata* is under consideration as a likely candidate for in situ

biomonitoring. *C. fornicata* survive well in field cages and reproduce frequently and prolifically with easily observable reproductive behavior. They can, therefore, be used for transplanting and for reproductive tests, directly indicating the onsite population level effects of a marine discharge.

Biomarkers (sublethal biochemical measures of effect) may serve as subcellular indicators of exposure to toxicants. In this context, biomarkers are defined as "molecular biological techniques that may directly link specific chemicals or classes of chemical compounds to observed biological effects." Two such biomarkers are measurements of increased incidence of sister chromatid exchange and increased synthesis of metallothionein.

Sister chromatid exchange is the exchange of pieces of DNA between the two arms of a chromosome. The rate of this exchange has been shown to increase in the presence of genotoxic agents (28). Even chemicals that do not directly affect DNA (nongenotoxic agents) may still affect the complicated mechanism of sister chromatid exchange, and, therefore, may change the exchange rate. This method will be explored further as an indicator of pollution effects on invertebrate and fish species.

Metallothionein induction is another measure of toxic exposure. Metallothionein is an intracellular metal-binding protein. In mammals and fish, the binding action induced by this protein may be a mechanism for tolerance to trace metals. Synthesis of the protein is induced by the presence of metals such as copper and cadmium. This and other "stress proteins" show promise as indicators of exposure to specific chemicals (28,6).

The winter flounder *Pseudopleuronectes americanus* is the first teleost organism studied in this program, and another candidate for population-level monitoring. This benthic fish species returns annually to its nursery grounds for spawning, making it a suitable indicator of adverse population effects due to pollutant exposure. In winter flounder, simple morphometric measures may indicate exposure to certain pollutants. Moore and Stegeman (40) have found liver tumors and severe nonneoplastic changes in the liver, and suggest that these are the result of exposure to aromatic hydrocarbons and chlorinated pesticides.

Integrated Approach for Toxic Discharge Control - Integration with Large-Scale Coastal Monitoring

The integrated approach of combining short-term CETTP tests with in situ biomonitoring not only promotes toxic discharge control efforts, but also can determine environmental trends. This integrated approach, therefore, might be integrated with large-scale coastal monitoring systems. A variety of Federally funded, regional monitoring systems are compiling and integrating vast amounts of chemical and biological environmental data to better define present environments and document trends that occur within them. NOAA's Status and Trends Program and EPA's Environmental Monitoring and Assessment Program (EMAP) are both designed for this purpose.

The Integrated Approach - Field Tested

An example of the combined use of CETTP and in situ biomonitoring tests is the New Bedford Harbor Pilot Dredging Project (Section 5). In the late 1970s, the Mussel Watch Program identified the harbor as a "hotspot" of PCB contamination. During the pilot project, biomonitoring was employed to monitor potentially toxic releases from the dredging and disposal operation.

The Superfund study also demonstrated the effectiveness of the real-time monitoring approach. In routine monitoring efforts, the time gap from data collection to analysis hinders progress, or renders the data meaningless when operations proceed before data analysis. In the New Bedford Harbor study, decision criteria were developed to help managers interpret and act on the monitoring data. Immediate decisions were made based on measurements that were taken less than 24 hours earlier.

Finally, the New Bedford Harbor pilot study showed the efficiency of the testing hierarchy - from mussel tissue residues to in situ tests to extensive lab and field biomonitoring. The hierarchy ensured that resources were conserved while the harbor characterization was completed. The case study in New Bedford Harbor stands as a model of the integrated approach for toxic discharge control.

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Appendix A.
Case Studies

CETTP Case Study I: Fernandia Beach, Florida

Toxicity testing of a complex effluent discharged into the Amelia River north of Jacksonville, Florida, was a cooperative effort between EPA Region IV (Athens, GA); the EPA Environmental Research Laboratory in Duluth, Minnesota (ERL-D); and ERL-N. The focus of the study was a point-source discharge from a pulp and paper mill in Fernandia Beach, Florida. The mill discharged approximately 16 million gallons (60 million liters) of effluent each day into the Amelia River with the outgoing tide. The case study was a week-long analysis of the effluent and its effects on the surrounding ecosystem. The Amelia River, a high-salinity tidal basin, proved to be a good location for a field evaluation of the Marine/Estuarine CETTP test methods. The goals of this study were:

- To determine the absolute toxicity of the pulp and paper mill effluent discharged at the site.
- To determine the relationship between effluent and receiving water toxicity.
- To generate data for deriving an NPDES discharge permit for the mill.

Dye studies, toxicity tests, and chemical analysis of the effluent were used to determine the source of the observed effects of the pulp-mill effluent.

Effluent Tests

Effluent samples were collected daily from the pulp mill's effluent lagoon adjacent to the discharge point. The samples were composited over a 24-hour period and held on ice until they were used in the toxicity tests. The following test methods were performed using the procedures specified in the EPA methods manual (8):

- A 7-day red macroalga (*Champia parvula*) reproductive test.
- A 7-day reproductive, growth, and survival test with the mysid *Mysidopsis bahia*.
- Tests using the inland silverside (*Menidia beryllina*) and sheepshead minnow (*Cyprinodon variegatus*) to measure larval fish growth and survival (41).

The effluent was toxic to all test species at concentrations higher than 32 percent; however, the receiving water concentrations of effluent in the river did not exceed 2 percent. The red macroalga and mysids were most sensitive to the effluent; the NOEC for the alga was 1 percent effluent, and the mysid's NOEC was 3.2 percent effluent. Fish larval growth was not significantly affected at any effluent dilutions. Survival, however, was reduced at 32 percent effluent for the sheepshead minnow, and at 10 percent for the inland silverside (see Table A-1).

Ambient Receiving Water Tests

Water samples were collected daily from 7 sampling stations in the river (except 1 day, when 21 stations were sampled; Figure A-1) during ebb tide, to coincide with the discharge. The following toxicity test methods were performed on the receiving waters (8):

- 7- to 9-day reproductive tests (performed on all 21 samples) with the red macroalga *Champia parvula*.
- A 7-day reproduction, growth, and survival test with the mysid (*Mysidopsis bahia*).
- A 7-day growth and survival test with the sheepshead minnow (*Cyprinodon variegatus*).

Using dye studies performed by EPA Region IV, estimates were made of the effluent concentrations in the receiving water samples collected (see Table A- 2). The receiving water concentrations of effluent in the river did not exceed 2 percent effluent, as was predicted by the dye studies. Only the red macroalga and mysids indicated toxicity at several monitoring stations. The alga was especially sensitive to several receiving water stations, and cystocarp production was reduced to zero or slightly above zero (indicating that the species was incapable of reproduction). The mysid receiving water tests were inconclusive because receiving water toxicity could not be correlated with estimated effluent toxicity based on the dye study. Finally, the sheepshead minnow showed no evidence of toxicity at concentrations as high as 10 percent effluent. The macroalga and sheepshead minnow tests showed a correlation between laboratory and field results (42).

Probable Causes of Toxicity

Using fractionation methods, EPA scientists at ERL-Duluth and Region IV were able to identify ammonia as the toxic constituent in the discharge. The freshwater crustacean *Ceriodaphnia dubia* showed toxic response to

Table A-1. Effects of Pulp Mill Effluent on Test Species (41)

Percent Effluent	Red Algae Mean No. Cystocarps	Mysid (%) Survival	Inland silverside (%) Survival	Sheepshead minnow (%) Survival
Control	11 ± 21	100	87	98
0.5	12 ± 3			
1.0	13 ± 1	100	80	98
2.5	0.2*			
3.2		88	58**	
5.0	0*			
10.0	0*	41*	33*	93
32		0*	0*	11*
50		0*	0*	0*

*Significantly different from control.
**High variability between three replicates, but not significantly different from the control.

the ammonia in the effluent. Other tests, using the red macroalga in effluent and ammonia mixtures containing the same concentration of the chemical, produced a very similar toxic response (see Figure A-2). The calculated concentrations of un-ionized ammonia (NH_3) in the effluent, along with the results from unpublished laboratory studies also point to ammonia as the cause of toxicity to the mysid and inland silverside (see Table A-3) (42). Ammonia also exhibits a characteristic concentration curve as ionization is affected by pH, temperature, and salinity.

Setting Permit Limits

Numerical, nonbinding permit limits were calculated using acute end-of-pipe toxicity data from the discharger's records, along with the NOECs determined during the study. Since the scientists were able to trace the toxicity to a specific chemical, and the toxicity was found at the very edge of the mixing zone set by the State of Florida, it was relatively simple to direct the discharger on how to reduce the effluent's toxicity. The State of Florida granted the pulp and paper mill a 3-year variance to the water quality standard, allowing them time to bring the effluent within permit limits. The EPA Region IV office will follow up on the toxicity testing at the end of the 3-year waiver.

CETTP Case Study II: East Greenwich, Rhode Island

Greenwich Cove is an estuarine cove located in Narragansett Bay between East Greenwich, Rhode Island, and Goddard State Park in Rhode Island (see Figure A-3). A Publicly Owned Treatment Works (POTW) in East Greenwich discharges an average of 0.7 million gallons per day (MGD) into the cove. At the time of the first phase of this case study, the POTW was designed to treat only 0.5 MGD. The Town of East Greenwich is currently upgrading the plant, and after modification, the plant will be capable of providing advanced secondary treatment for 1.2 MGD of sewage. A variety of marine/estuarine CETTP tests were used to determine the effluent and receiving water toxicity for the cove during four field studies performed over a 1-year time period (December 1985, July, September, and November 1986).

After the POTW is upgraded, additional toxicity tests will be conducted, providing a "before and after" picture of the effluent's toxicity and its effects on the cove.

Chemical and Physical Effluent Characterization

Chemical Characterization. The POTW effluent was analyzed for dissolved and particulate trace metals (cadmium, chromium, copper, iron, lead, manganese, nickel, zinc); organic compounds such as PCBs and PAHs; and nutrients (e.g., ammonia, nitrogen, and phosphorous). Total suspended solids and total residual chlorine also were measured. Effluent samples were collected for fractionation studies to determine what class(es) of compounds caused the observed toxicity. As part of the fractionation procedure, toxicity tests were conducted. These tests showed that chlorinated effluent was more toxic than unchlorinated, and that the majority of the toxicity could be attributed to a compound in the dissolved portion rather than one associated with the particulates. When further separation of the effluent was performed, it was determined that the toxicity was probably due to moderately polar constituents (43).

Physical Characterization. Mixing in the cove was determined by dye study techniques. The dye studies also provided information on the age of the effluent at the different sampling stations in the cove (see Figure A-4). At the center of the boil area (the area above the submerged outfall, or where the effluent actually enters the water from the discharge pipe) effluent concentration was measured at as high as 40 percent. The concentration dropped to less than 1 percent, however, at all other sampling stations. Old effluent (effluent remaining

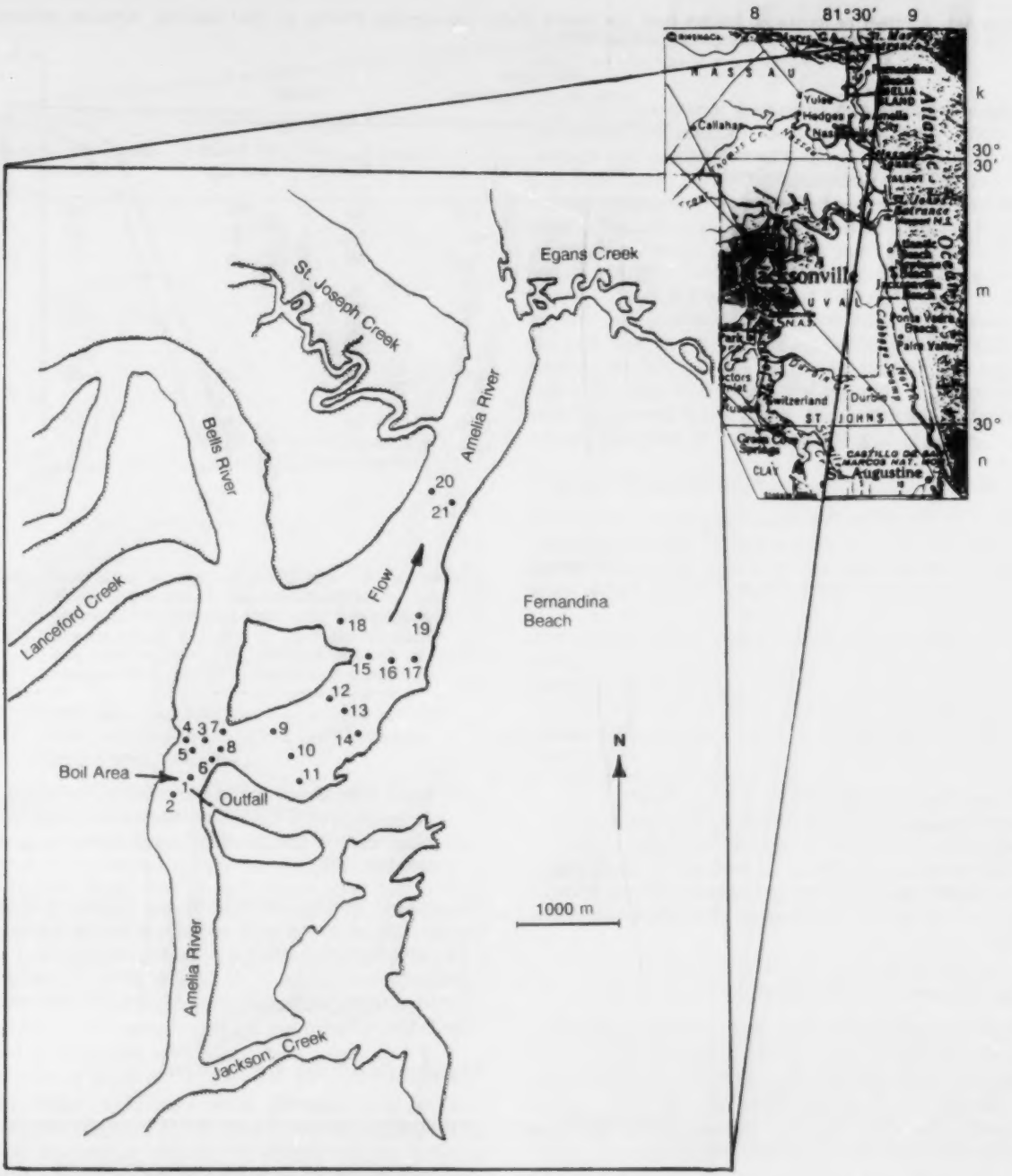

Figure A-1. Amelia River, in Fernandina Beach, Florida (42).

in the cove after one tidal cycle) and new were found to coexist in the cove - the mixture near the outfall was primarily fresh effluent (Station 2); older effluent was found near the mouth of the cove (Stations 4 and 5); and the oldest effluent was found at the head, or southernmost extent of the cove (Station 1). Figure A-5 shows the mixing characteristics as determined using dye studies.

No permanent (more than 2-day) stratification in temperature, salinity, or dye concentrations was detected in the study, although a salinity gradient was measured that showed increased salinity from the interior of the

Table A-2. Effect of Receiving Waters from the Amelia River, Jacksonville, Florida, on Test Species. Effluent percents were calculated based on a dye study (42).

Station Number	Percent Effluent	Red Algae Mean No. of Cystocarps		Mysid		Sheepshead Minnow (5) Survival
		May 17	May 18	(%) Survival	(%) Females with Eggs	
1	2.0	0.5*	0.8*	90	64	98
2	control (0%)	14.7	19.0	93	71	98
3	- (no data)	1.0*	3.0*	88	77	100
4	1.5		10.4			
5	-	2.3*	0.6*	98	47	100
6	0.05		1.1*			
7	1.4		3.4			
8	0.08	8.3	21.0	93	14**	95
9	1.4		1.1*			
10	0.5	15.2	13.5	93	66	100
11	0.2	12.8	19.9	93	31	100
12	0.9					
13	0.7		7.2			
14	0.5		5.3*			
15	0.5		5.2*			
16	-		7.9			
17	0.6		11.7			
18	0.5		9.7			
19	0.4		10.4			
20	0.4		10.2			
21	0.5		9.6			

*Significantly different from control.
**Significantly different from control, but not attributed to effluent toxicity.

cove (where the freshwater Maskerchugg River contributes to the cove) to the outer cove, toward Greenwich Bay. Table A-4 shows the flow rates in the cove. Tidal range and currents, as well as the discharge flow rate of both the POTW and the Maskerchugg River, all affect the mixing and stratification characteristics in the cove (43).

Complex Effluent Toxicity Tests

The chlorinated effluent samples were diluted to effluent concentrations ranging from 32 to 3.2 percent with relatively clean Narragansett Bay seawater, and the salinity was adjusted to a standard of 30 parts per thousand with Narragansett Bay brine (8). The following tests were performed on the effluent samples:

- Sperm cell toxicity tests with the sea urchin *Arbacia punctulata*.
- Reproductive effect tests on the red macroalga *Champia parvula*.
- 7-day reproduction, survival, and growth tests using *Mysidopsis bahia*, the mysid.
- Tests with sheepshead minnows (*Cyprinidon variegatus*) and the inland silverside (*Menidia beryllina*) to indicate effects on fish larvae growth and survival.

The effluent toxicity varied from day to day. Averaging the toxicity over all of the test periods, a concentration of 0.24 percent of the effluent affected sea urchin reproductive capacity, while greater than 10 percent effluent concentration was toxic to the fish species tested (see Table A-5).

Ambient Receiving Water Tests

The scientists used the same methods to test the toxicity of the effluent as were used in the complex effluent analysis, and an additional test was performed, the kelp reproduction test. This test uses the sea kelp *Laminaria saccharina* (see Section 2) to evaluate the reproductive effects of the receiving water.

All samples from the boil station showed similar effects on the test species. The other stations gave variable results. Percent effluent at each station was determined using dye studies (Table A-6). The species' toxic responses differed from one test period to the next, indicating a highly

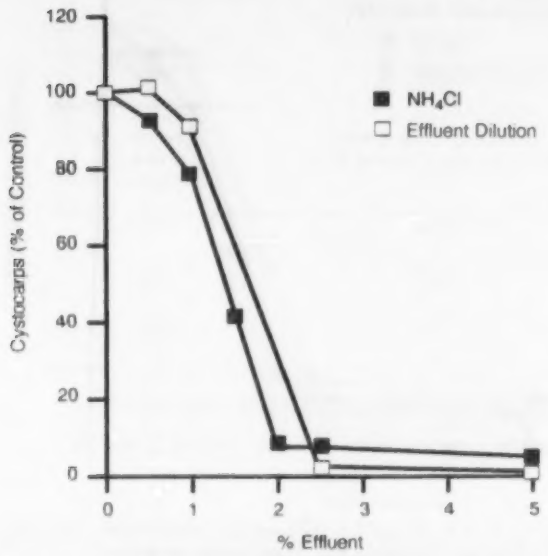

Figure A-2. **Number of cystocarps (as percent of the number on control-exposed red microalga) versus percent effluent. The effluent data are averaged over 2 days. A stock solution of 26.4 mg NH_4Cl/100 mL was used to match the effluent with an assumed concentration of 70 mg NH_4-N/L of effluent (42).**

variable effluent. The red macroalga *Champia* and the sea urchin were most sensitive to the contaminated receiving waters. (see Table A-7).

Conclusion

The East Greenwich Cove Marine CETTP was generally successful in its attempt to field-validate the laboratory effluent toxicity tests. The investigators found that effluent "residence time" and toxicity half-life (or rate of toxicity reduction) are important factors to build into estimations of lab to field results. In this case, the variable receiving water toxicity could be explained by the variability in the effluent coming from the POTW, and by toxicity reduction or degradation with time. Although the toxicity could not be traced to a specific chemical, it was concluded that the total toxicity observed in the field generally could be attributed to the POTW discharge, although occasional toxicity observed in the more sensitive tests distant from the discharge could be from other sources (e.g., boats moored in the cove, runoff, and contaminants in the Maskerchugg River, etc.).

CETTP Case Study III: Panama City, Florida

This study in Saint Andrew Bay (see Figure A-6) focused on the effluent from the Bay County Wastewater Treatment Plant. The plant discharges about 30 million gallons of waste per day into the bay. Scientists from EPA Region IV (Athens, Georgia) used dye studies, physical and chemical characterization of the effluent, and CETTP testing methods to determine the discharge's effect on the bay's ecosystem.

A 25-hour dye tracer study was conducted to establish the mixing and dilution characteristics of the effluent. The release was continuous with the discharge and spanned one tidal cycle. This initial measurement provided concentrations for the receiving water tests, and further measurements over five additional tidal cycles provided information on the effluent buildup in the bay. Table A-8 shows the dilutions in the bay at the time of the study. Figure A-7 shows the dilutions averaged over six tidal cycles. The scientists concluded that those areas east of the boil receive effluent during flood tides, while those west of the boil receive maximum exposure during ebb tide (44). They also found that the effluent's concentration was maximized in the upper 1 to 3 meters of the water column because of its lower density.

Effluent Characterization and Toxicity Testing

Measures of biological oxygen demand (BOD_5), total suspended solids (TSS), and fecal coliform counts were performed on the discharged wastewater, and the results were within the POTW's reported values.

The toxicity of the freshwater effluent was determined using a larval survival and growth test with the freshwater species *Pimephales promelas*, or fathead minnow, and a survival and reproduction test using *Ceriodaphnia dubia*, a freshwater crustacean. The effluent was made saline (using commercial salt mix or hypersaline brine) for the following marine organism tests:

- Static acute toxicity test using the mysid *Mysidopsis bahia*.
- Sperm cell toxicity test with the sea urchin *Arbacia punctulata*.
- Reproduction and toxicity test using the red macroalga *Champia parvula*.

The results of these tests are summarized in Table A-9. The freshwater tests with 100 percent effluent showed no effect on daphnid or larval fathead minnow survival and growth. Reproduction in the daphnid was affected, however, at 100 percent effluent. The No-Observed-Effect Concentration (NOEC) in this case was 30 percent. The effluent was acutely toxic to the mysids, with a LC50 concentration of 70.7 percent. Sea urchin fertilization was unaffected at 70 percent effluent, the highest concentration tested. The NOEC for the red macroalgal reproduction was 12 percent effluent.

Receiving Water Toxicity Tests

Receiving water samples were collected in June and July, 1988. In June, four samples were collected at each station (boil, 200E, and 200W) in a consecutive ebb,

Table A-3. Toxicity Comparisons of Calculated Un-ionized Ammonia in Effluent (Average Concentration was Calculated from Total Ammonia, which was 3.66 mg/L) and Separate Ammonia Tests (42)

	Effluent Tests		Ammonia Tests**
Species	Lowest Effect Concentration (% Effluent)	Calculated Chronic Value* for Un-ionized Ammonia (NH), (mg/L)	Chronic Value (mg/L)
Champia parvula (red macroalga)	2.5	0.06	0.04
Mysidopsis bahia (mysid)	3.2	0.07	0.12
Menidia beryllina (inland silverside)	10.0	0.20	0.06

*Chronic value is the geometric mean of the highest no-effect concentration and the lowest effect concentration for 2-day tests (*C. parvula*) and 7-day tests (*M. bahia* and *M. beryllina*).
**Unpublished ERL-N data; chronic numbers refer to calculated un-ionized ammonia concentrations from 2-day *M. bahia* tests, and 28-day *M. beryllina* tests.

Greenwich Cove in winter (ice in foreground).

slack low, flood, and slack high tide. Grab samples were collected near the boil, Shell Island, and station 1E for the July test period (Figure A-8). The same marine species tests were performed as for the effluent tests. ERL-N assisted Region IV, performing the sea urchin and algal tests in the Narragansett lab. At the site, dissolved oxygen, salinity, and temperature measurements were taken, as well as light transmission readings. Figure A-9 shows the light transmission at four stations; Table A-10 gives the salinity, temperature, and dissolved oxygen measurements for the same time period.

The mysids showed no response to the receiving water at any of the stations. The red macroalga reproductive tests were inconclusive; the varied results suggested that the St. Andrew Bay waters are in themselves unable to support *Champia parvula* reproduction (see Table A-11).

A survey of the effluent's effects was performed on the bay's seagrass community. That study is described in Dilution Study and Toxicity Testing, Bay County Wastewater Discharge (44).

In Situ Biomonitoring Case Study: East Greenwich, Rhode Island

An in situ biomonitoring study was conducted in Greenwich Cove in Greenwich Bay, Rhode Island, during October and November, 1986, to compare the results of scope for growth tests on mussels (Mytilus edulis) conducted over long and short time periods. The short-term test (7 days) was conducted within the time period of the long-term, 30-day test. The observed toxicity could be attributed to the East Greenwich POTW.

Method

Three mussel deployment stations were located around the cove: one was in the cove near the outfall (Station 3); the second was in the mixing zone between the cove and Greenwich Bay (Station 6); and the third was at the mouth of Greenwich Bay (Station 10; see Figure A-10). A reference station (REF) was chosen at the mouth of Narragansett Bay. As described in Section 3, polypropylene baskets containing mussels were anchored at each station, suspended 1 meter above the bottom. Clearance rate, respiration rate, and food assimilation efficiency were measured as described in Section 3 of this document. Average scope for growth was calculated for each group of mussels, and the 7- and 30-day values were compared for each station (27).

Results

There was a graded response in scope for growth of the mussels exposed for both 7 days and 30 days. Table A-12 shows the SFG and individual parameter results at each station, and compares the 7-day and 30-day exposure times. The SFG of the mussels at the reference

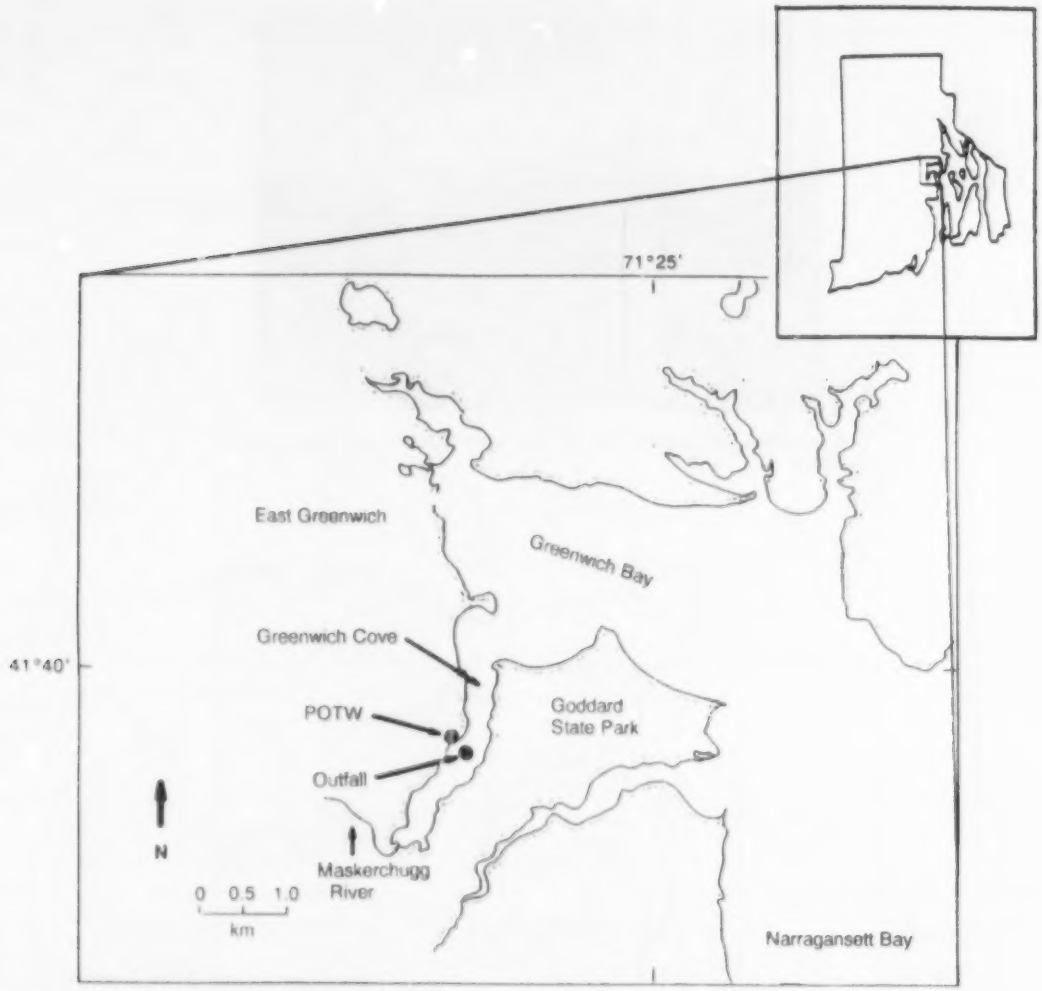

Figure A-3. Greenwich Cove, Rhode Island (43).

station was almost unchanged from 7 to 30 days. SFG at the remaining stations, however, decreased moving toward the East Greenwich POTW outfall with both time periods. This trend in SFG was more pronounced after 30 days of exposure (27). The more dramatic decrease observed after 30 days may indicate continued physiological decline with prolonged exposure to the effluent, but since actual effluent exposure was not measured concurrently, the physiological decline cannot be tied conclusively to the effluent's presence in the cove. Tissue residue analysis is being conducted, and will provide more information about the conditions of the cove waters during the exposure period.

The results of this study imply that the effects of 7-day exposure are indicative of longer-term effects, and can be used to predict biological response to effluent toxicity.

East Greenwich POTW, viewed from Greenwich Cove (December 1985).

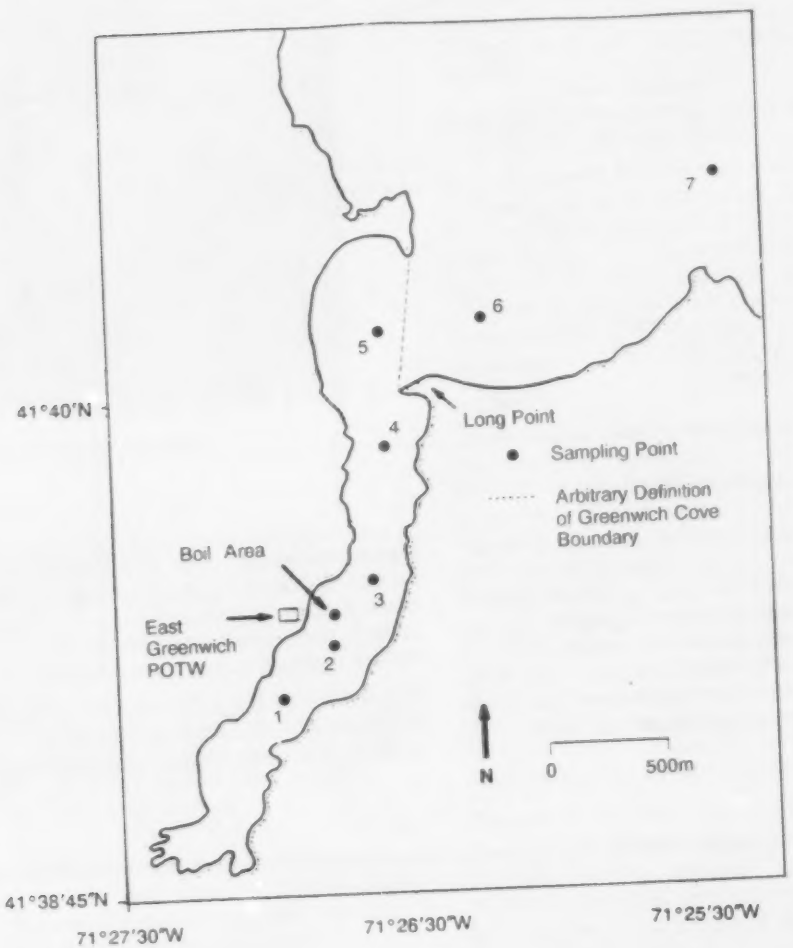

Figure A-4. Sampling stations in Greenwich Cove (43).

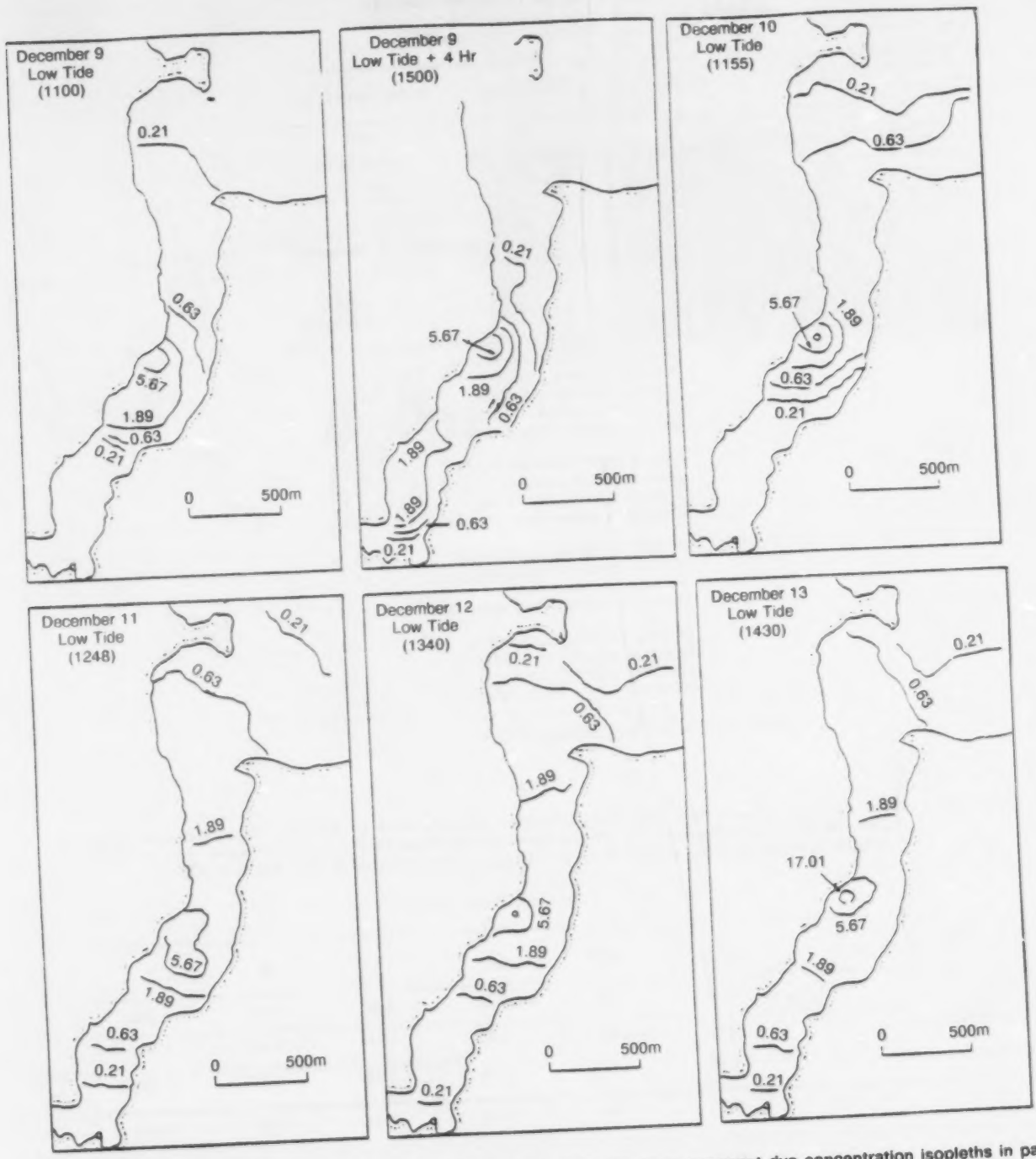

Figure A-5. Dye distribution in Greenwich Cove during December, 1985. Numbers represent dye concentration isopleths in parts per billion (ppb) (43).

Table A-4. Flow Rates into Greenwich Cove (43)

Source	Flow Rate (m^3/day)	Flow Rate (million gallons/day)
POTW	2,650	0.70
Maskerchugg R.	1,420 to 138,000	0.38 to 36.46
Mean Tidal Flow	2,300,000	607.70

Table A-5. Rank Order of Species Sensitivity to Greenwich Cove POTW Effluent (43)

Study Period	Species	NOEC* (% Effluent)
12/85	*Arbacia punctulata* (sea urchin)	1.0
	Mysidopsis bahia (mysid)	1.0
	Champia parvula (red algae)	3.0
	Cyprinodon variegatus (sheepshead)	10.0
	Menidia beryllina (inland silverside)	10.0
7/86	*Arbacia punctulata* (sea urchin)	< 0.3
	Mysidopsis bahia (mysid)	3.2
	Champia parvula (red algae)	5.4
	Menidia beryllina (inland silverside)	10.0
9/86	*Arbacia punctulata* (sea urchin)	< 0.24
	Champia parvula (red algae)	4.25
	Mysidopsis bahia (mysid)	10.0
	Menidia beryllina (inland silverside)	10.0
11/86	*Arbacia punctulata* (sea urchin)	< 0.6
	Champia parvula (red algae)	2.5
	Mysidopsis bahia (mysid)	3.2
	Menidia beryllina (inland silverside)	10.0

*NOEC = No-Observed-Effect Concentration.

Table A-6. Mean Percent Effluent from the East Greenwich POTW Found in Surface Water Samples from Greenwich Cove and Greenwich Bay, Rhode Island During Three 1986 Sampling Periods: July 16 to 22, September 8 to 15, and November 9 to 18. All sampling was performed at slack low tide (43)

Date	Receiving Water Stations 1	2	outfall	3	4	6	7
July	0.16	0.15	26	0.28	0.35	0.12	0.064
September	0.13	0.15	29	0.30	0.30	0.12	0.054
November	0.23	0.28	17	0.38	0.24	0.27	0.083
Overall MEAN	0.17	0.18	24	0.33	0.30	0.16	0.063
Overall MAXIMUM	0.33	0.73	40	0.66	0.68	0.86	0.14
Overall MINIMUM	0.031	0.010	6.3	0.082	0.14	0.039	0.013

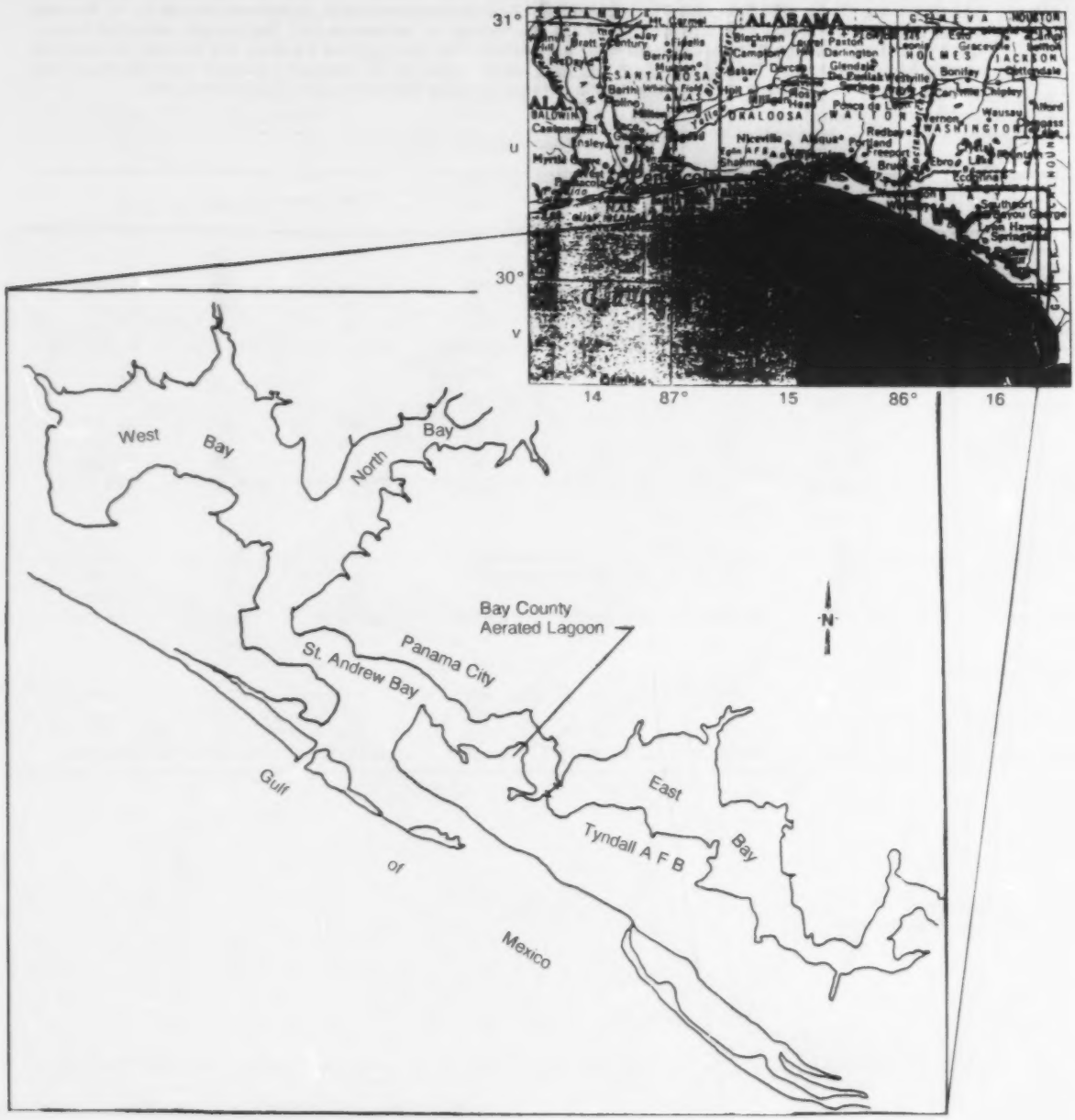

Figure A-6. St. Andrew Bay, Panama City, Florida (44).

Table A-7. **Results of Toxicity Tests Conducted on Surface Waters from Greenwich Cove and Greenwich Bay, Rhode Island, During Three 1986 Sampling Periods: July 16 to 22, September 9 to 15, and November 11 to 18. Toxicity is expressed in the numerator of each fraction as the number of occasions that the sample collected from a specific station was significantly different from the control. The denominator denotes the number of receiving water samples evaluated. Overall toxicity is the total number toxic of all samples collected from the three test periods; the number in parentheses denotes the percent samples that were toxic of all tested (43).**

Species/Date	Receiving Water Stations						
	1	2	outfall	3	4	6	7
C. parvula (red algae)							
July	0/6	0/6	6/6	0/6	1/6	1/6	1/6
September	0/5	0/5	5/5	0/5	0/5	0/5	0/5
November	1/1	1/1	1/1	1/1	1/1	0/0	1/1
Overall Toxicity	1/12 (8%)	1/12 (8%)	12/12 (100%)	1/12 (8%)	2/12 (17%)	1/12 (8%)	2/12 (17%)
A. punctulata (sea urchin)							
July	1/7	5/7	7/7	3/7	4/7	3/7	4/7
September	4/6	3/6	6/6	3/6	1/6	1/6	1/6
November	2/6	1/6	6/6	2/6	2/6	2/6	2/6
Overall Toxicity	7/19 (39%)	9/19 (47%)	19/19 (100%)	8/19 (42%)	7/19 (37%)	6/19 (32%)	8/19 (42%)
M. bahia (mysid)							
July	0/1	0/1	1/1	0/1	0/1	0/1	0/1
September	0/1	0/1	1/1	0/1	0/1	0/1	0/1
November	0/1	0/1	1/1	0/1	0/1	0/1	0/1
Overall Toxicity	0/3 (0%)	0/3 (0%)	3/3 (100%)	0/3(0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
M. beryllina (inland silverside)							
July	0/1	0/1	1/1	0/1	0/1	0/1	0/1
September	0/1	0/1	1/1	0/1	0/1	0/1	0/1
November	1/1	0/1	1/1	0/1	0/1	0/1	0/1
Overall Toxicity	1/3 (33%)	0/3 (0%)	3/3 (100%)	0/3(0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)

Table A-8. Dilution Summary, St. Andrew Bay, Florida, June, 1988 (49)

Station	Low Tide		High Tide		Average Conditions Wastewater Dilution
	Ultimate Tracer Conc.*	Wastewater Dilution	Ultimate Tracer Conc.*	Wastewater Dilution	
10E	0.9	800:1	0.6	1200:1	1000:1
8E	1.3	550:1	2.0	360:1	455:1
6E	2.1	340:1	2.1	340:1	340:1
4E	2.1	340:1	2.2	325:1	335:1
2E	2.5	285:1	5.3	135:1	210:1
200E	10.8	66:1	18.3	39:1	53:1
Boil	45.5	16:1	40.0	18:1	17:1
200W	15.0	48:1	16.2	44:1	46:1
2W	4.3	165:1	3.4	210:1	190:1
4W	4.0	180:1	2.2	325:1	255:1
6W	4.1	175:1	1.4	515:1	345:1
8W	3.2	225:1	0.9	800:1	510:1
10W	2.7	265:1	0.2	3600:1	1930:1
12W	0.8	900:1	0.6	1200:1	1050:1
4S	1.4	515:1	0.2	3600:1	2050:1

*ppb

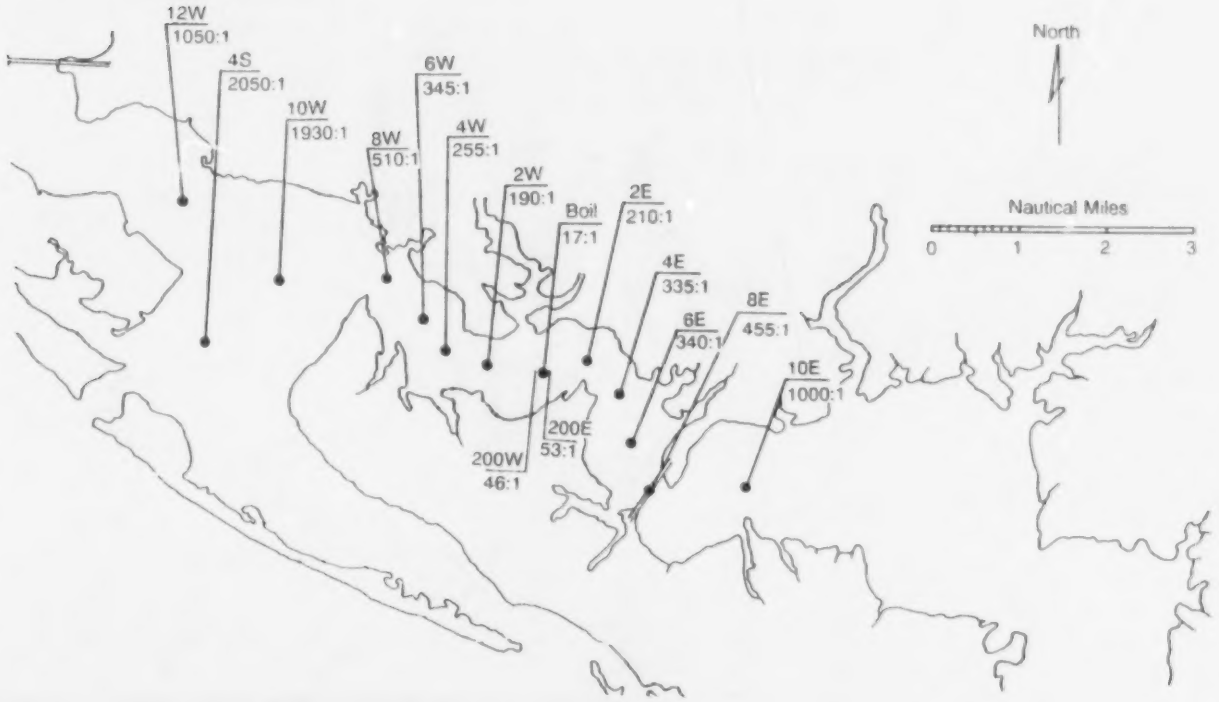

Figure A-7. Steady-state dilution, average conditions, June 1988 (49).

Table A-9. Toxicity of Effluent from Bay County Wastewater Treatment Plant, Panama City, Florida (44)

Percent Effluent	Daphnid (%) Survival	Daphnid Mean No. Offspring/Female	Mysid (%) Survival[1]	Fathead minnow (%) Survival	Sea urchin (%) Fertilization	Red Algae Mean No. Cystocarps
0[2]	100	26.47	100	100	95.5	9.0
1	100	17.63	100	100		
3	100	28.60		100		
6						6.6
10	100	30.43	100	100		
12						5.4*
25			80			2.4*
30	100	28.83		100		
50			80			1.0*
70					89.6	
100	90	5.00*	20*	100		0*

[1]The calculated LC50 for *Mysidopsis bahia* was 70.7 percent.
[2]Control sample collected from Narragansett Bay.
*Significantly different from control.

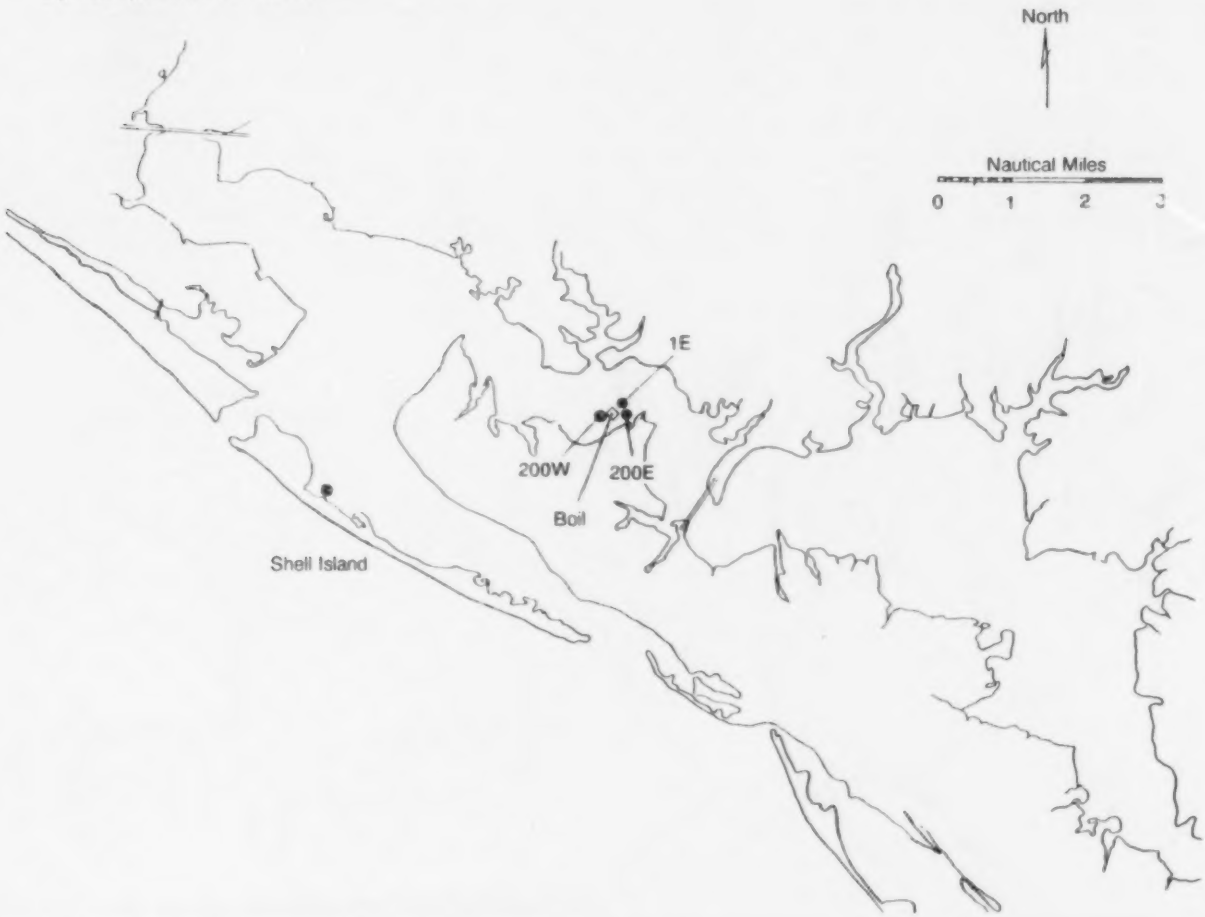

Figure A-8. St. Andrew Bay receiving water sampling stations (44).

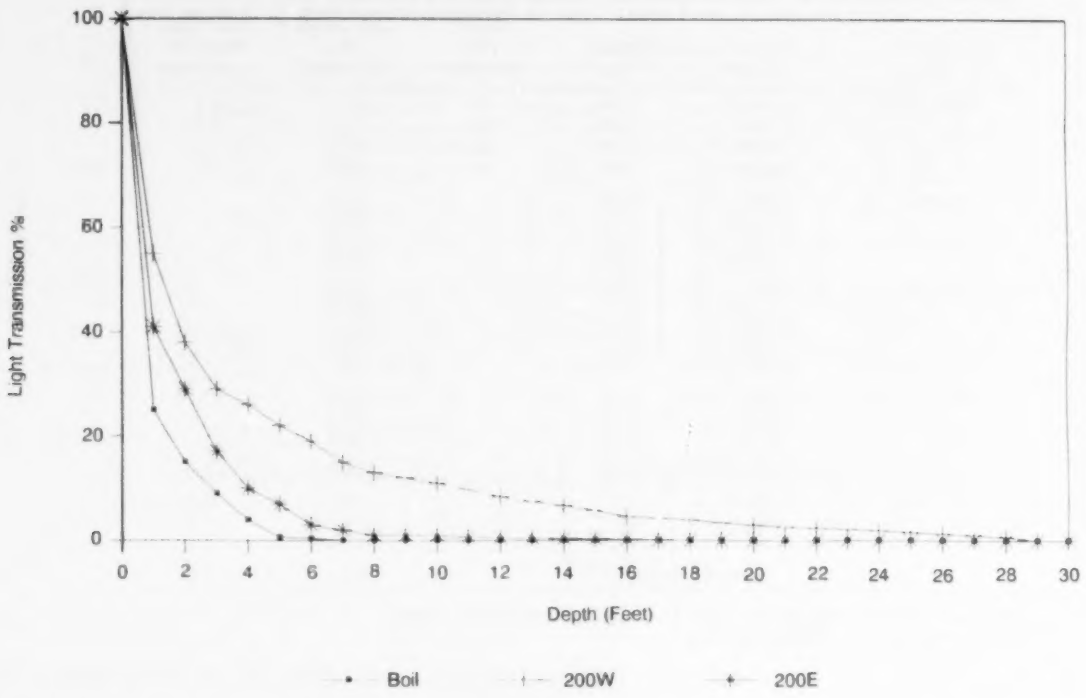

Figure A-9. Light transmission, as measured by marine photometer (44).

Table A-10. Salinity, Temperature, and Dissolved Oxygen at the Boil, 200E, and 200W Stations in June, 1988 (44)

	Depth (ft.)	Sal. (ppt)	Temp. (°C)	D.O. (ppm)
Boil	1	32.3	26.9	6.0
	5	32.9	27.6	6.2
	9	32.8	27.2	6.5
	15	33.6	26.6	6.4
	19	34.9	26.8	6.5
	25	35.0	26.6	6.5
			Avg. =	6.4
B-200E	1	32.9	28.0	6.2
	5	32.9	27.2	6.0
	9	33.5	27.3	6.3
	15	34.6	27.0	6.3
	19	35.2	26.4	6.1
			Avg. =	6.2
B-200W	1	32.5	28.1	6.9
	5	32.5	28.0	7.0
	9	33.0	27.4	6.9
	15	33.4	27.0	6.8
	19	34.1	26.4	6.3
	25	34.4	26.4	6.4
			Avg. =	6.6

Table A-11. Results of *Mysidopsis bahia* (mysid), *Arbacia punctulata* (see urchin), and *Champia parvula* (red algae) Tests on Receiving Waters from St. Andrew Bay

Sample Date	Sample Location	Sample Time	Mysid (%) Survival	Sea Urchin (%) Fertilization	Red Algae Mean No. Cystocarps
6/14-15/88	Boil	1130	100	92.8	2.9
	50W	1325	100	89.4	1.7*
	100W	1220	100	87.9	4.5*
	200W	1305	90	88.8	2.7
7/15/88	Boil	2050		81.3	
	50W	2110		86.9	
	50E	2145		84.5	
	100E	2130		86.5	
6/15/88	Boil	0800		86.0	
	50E	0815		82.8	
	100E	0830		93.5*	
	200E	0840		89.9	
	Boil	1100		87.7	
	100W	1110		83.2	
	200W	1125		86.2	
	Control	Treatment[1]		95.5	13.9
7/19/88	Boil	1321		96.0	6.8
	Shell I.	1307		94.7	1.1*
	1E	1347		93.4	3.3
	Control	Treatment[1]		97.4	9.0

*Significant difference from the boil based on Analysis of Variance and tests.
[1]Sample collected from Narragansett Bay.

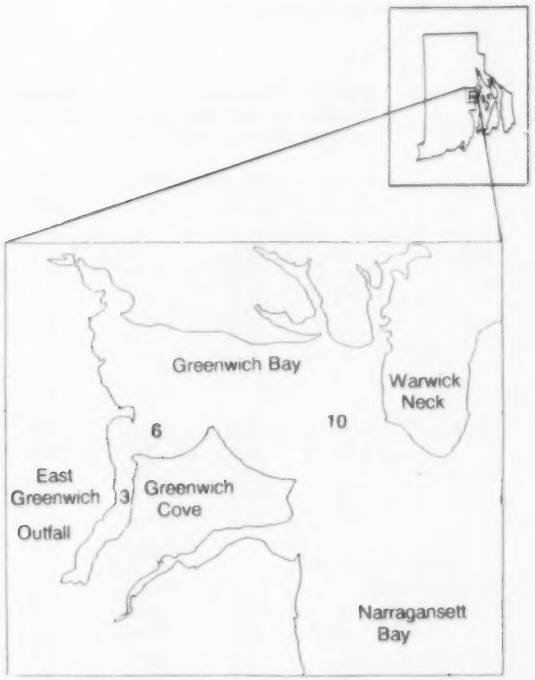

Figure A-10. Greenwich Cove, Rhode Island, with monitoring stations (27).

Table A-12. Results of the Physiological Measurements and Scope for Growth Index of Mussels from 7- and 30-Day Exposure (Values in Parenthesis are Standard Errors) (27)

	7-Day Exposure			
Station	Clearance Rate (L/h)	Absorption Efficiency %	Respiration Rate (mL-02/h)	Scope for Growth (J/h)
Ref	5.11 (0.22) AB	85 (1) A	0.29 (0.03) A	17.7 (1.1) A
10	6.07 (0.23) A	91 (0) B	0.48 (0.04) B	17.7 (0.9) A
6	4.48 (0.28) B	89 (2) AB	0.50 (0.02) B	13.0 (1.7) AB
3	4.30 (0.39) B	86 (0) AB	0.50 (0.03) B	11.4 (1.3) B
	30-Day Exposure			
Station	Clearance Rate (L/h)	Absorption Efficiency %	Respiration Rate (mL-02/h)	Scope for Growth (J/h)
Ref	5.88 (0.58) A	84 (5) A	0.39 (0.04) A	17.2 (2.4) A
10	5.02 (0.46) AB	95 (0) B	0.53 (0.02) AB	15.0 (1.7) AB
6	4.41 (0.42) AB	96 (1) B	0.68 (0.08) B	10.7 (2.0) AB
3	3.05 (0.54) B	89 (1) AB	0.55 (0.06) AB	6.5 (3.2) B

Appendix B
Glossary

Absolute Toxicity. Laboratory-determined toxicity (usually determined using CETTP methods) of a complex effluent without comparison to receiving water toxicity.

Acute. Occurring within a short period of time. In toxicity tests, a response observed within 96 hours or less typically is considered an acute effect.

Acute to Chronic Ratio (ACR). The ratio of the acute toxicity of a substance (expressed as an LC50) to its chronic toxicity (expressed as a NOEC, LOEC, or EC50). ACRs can be used to estimate chronic toxicity on the basis of acute toxicity data, helping in the determination of NPDES permit limits.

Ambient Toxicity. Toxicity produced by a sample of water taken from a larger water body.

Benthic Zone. The ocean floor.

Bioaccumulation. The uptake and retention of substances by an organism from its surrounding media and food.

Bioavailability. The degree to which a substance is or becomes available to the tissues after exposure. A substance's bioavailability determines its effect on exposed organisms.

Biochemical Oxygen Demand (BOD). The quantity of oxygen utilized in the biochemical oxidation of organic matter in a specified time and at a specified temperature. It is not related to the oxygen requirements in chemical combustion, being determined entirely by the availability of the material as a biological food and by the amount of oxygen utilized by the microorganisms during oxidation.

Biomarker. A molecular biological indicator that may directly link specific chemicals or classes of chemicals to observed biological effects.

Biomonitoring. Any method of testing that includes the use of aquatic plant or animal species to measure acute or chronic toxicity, and any biological and/or chemical measure of bioaccumulation.

Biosurvey. A method for determining the variety and numbers of species in a biological community.

Chronic. Occurring after a prolonged period of time. A chronic effect is one that is observed after long-term (usually low-level) exposure.

Chronic Value (ChV). The geometric mean of the Lowest-Observed-Effect concentration (LOEC) and the No-Observed-Effect Concentration (NOEC).

Clearance Rate (CR). The volume of water that a mussel can completely "clean" (remove particles larger than 3 microns) within a specific time period.

Cystocarp. The spore case of the red marine alga *Champia parvula*. Cystocarps are evidence of sexual reproduction in the algae, and their formation is used as a toxicity test endpoint.

Diversity. The number of species in a specific location.

Direct Discharge. Release of a pollutant from a pipe or outfall directly into a water body.

Effective Concentration (EC). The effluent concentration that has an effect on a given percentage of test organisms.

Effective Concentration/50 (EC50). The effluent concentration that has an effect on 50 percent of the test organisms.

Effluent. Any pollutant or mixture of pollutants that is discharged into surface waters or treatment works.

Effluent Limitation. A restriction imposed by State or Federal authorities on the quantities, rates, and concentrations of pollutants that are discharged into surface waters.

Estuary. The lower end of a river where freshwater meets and mixes with seawater.

Fecundity. An organism's capacity for producing offspring.

Fluorometry. An instrumental method for measuring the amount of fluorescence in a water sample. Used during dye studies to estimate the concentration of an effluent in the receiving water.

Fractionation. A method for separating and identifying the components in a chemical mixture and each part's effects on test organisms. For example, an effluent may be gravity filtered, and the filtered solution used in a toxicity test.

Gas Chromatography (GC). An instrumental method for identifying the component parts (especially volatile

organics) in a chemical mixture. The solvent extract of the aqueous sample is injected into a column containing a stationary phase (resin) and a carrier phase (gas). The vaporized volatile components leach off the column according to their relative affinity for the stationary and carrier phases, and show peaks on a chart proportional to their presence in the mixture.

Hypersaline. A solution whose salinity is greater than normal seawater.

Larva. An immature form that is unlike the adult organism.

Lowest-Observed-Effect Concentration (LOEC). The lowest concentration of a substance at which an effect was observed in test organisms.

No-Observed-Effect Concentration (NOEC). The highest concentration of a substance at which no effect was observed in test organisms.

Nonpoint Source. A pollution discharge source that is not released from a distinct source, such as stormwater runoff that is collected and channeled by humans.

National Pollutant Discharge Elimination System (NPDES). The National program for administering permits for the discharge of pollutants into the waters of the United States under the Clean Water Act.

Outfall. The discharge point from any discernible conveyance for effluent discharge (i.e., a pipe, ditch, or channel).

Phylogenetic. Pertaining to ancestral development.

Point Source. A single source of effluent, such as a manufacturing facility, that discharges into surface waters.

Precision. When repeated test results do not deviate significantly among themselves. Compare this to accuracy, in which the test results do not vary significantly from a standard.

Relative Toxicity. The toxicity of an effluent after it is mixed in a receiving water - determined with either CETTP receiving water tests or in situ biomonitoring methods.

Scope for Growth. A measure of the energy available to an organism for tissue production, both somatic and reproductive, after routine metabolic costs are accounted for.

Superfund. Federal authority under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, ratified in 1980) to respond directly to releases or threats of releases of hazardous substances. "Superfund" refers to the Federal funds that are available to help pay for hazardous waste cleanups.

Teratogenicity. A substance's ability to produce anomalies of formation or development.

Wasteload Allocation. The portion of a receiving water's total maximum allowable daily pollution load that is allocated to one of its existing or future point sources of pollution.

Whole Effluent Toxicity. The aggregate toxic effect of an effluent (usually measured directly with a toxicity test).

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